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(54) Title: DIAGNOSTIC ASSAYS FOR PARVOVIRUS B19

(57) Abstract: Human parvovirus B19 primers and probes derived from conserved regions of the parvovirus B19 genome are disclosed. Also disclosed are nucleic acid-based assays using the primers and probes.

DIAGNOSTIC ASSAYS FOR PARVOVIRUS B19

Technical Field

The present invention pertains generally to viral diagnostics. In particular, the invention relates to nucleic acid-based assays for accurately diagnosing parvovirus B19 infection and to primers and probes for use in these assays.

Background Of The Invention

Human parvovirus B19 is a member of the family Parvoviridae, genus Erythrovirus and is a small 22-nm icosahedral nonenveloped virus with a linear single-stranded DNA molecule of approximately 5,600 nucleotides. The viral genome encodes three major proteins, VP1, VP2 and NS1. See, Shade et al., *J. Virol.* (1986) 58:921-936 and Figure 1 herein. VP1 (83kDa) and VP2 (58 kDa) are the structural proteins of the capsid. The two proteins are encoded in overlapping reading frames from about nucleotides 2444 to 4789 and about 3125 to 4789, respectively. VP2 constitutes 95% of the capsid and the larger VP1 protein only 5% of the capsid. VP1 is required for the mature conformation of the virus. NS1 (77 kDa), is a nonstructural protein and is present only in the nuclear fraction of infected cells and absent from the cytoplasm and intact virions in sera.

Parvovirus B19 was first discovered in the sera of normal blood donors and is the only member of the family Parvoviridae known to be pathogenic in humans. The virus is associated with a wide range of disease manifestations. Human parvovirus B19 normally causes an asymptomatic or mild self-limiting infection in children. In adults, parvovirus B19 may cause a rash, transient symmetrical polyarthralgia and arthritis. Parvovirus B19 has been associated with transient aplastic crisis (TAC) in

patients with underlying hemolytic disorders. Chronic B19 infection and persistent anemia have been reported in immunocompromised patients with acute leukemia, congenital immunodeficiencies, AIDS, and following bone marrow transplantation. Parvovirus B19 has also been associated with fetal death in pregnant women.

In most countries, B19 virus infection generally occurs during childhood, with approximately 50% of children having anti-B19 antibodies by the age of 15 years. B19 antibody prevalence may further increase during lifetime and reaches values higher than 90% in elderly individuals.

In human parvovirus B19 infection, initial viral replication is believed to occur in the respiratory tract. The virus then targets cells in the bone marrow. This leads to large-scale viral replication with reported viremia of between 10^2 to 10^{14} particles/ml, occurring 7-10 days after infection but prior to the onset of symptoms. Cessation of viremia coincides with the detection of specific IgM antibodies that remain elevated for two to three months. Anti-B19 IgG antibodies are detected a few days after IgM antibodies appear and persist lifelong.

The absence of a lipid envelope and limited DNA content make parvovirus B19 extremely resistant to physicochemical inactivation. Parvovirus B19, especially at high concentration, can withstand conventional heat treatment of blood products and transmission of B19 through the administration of solvent-detergent-treated factor VIII and steam- or dry-heated factor VIII and IX preparations has been documented.

Human parvovirus B19 cannot be grown in conventional cell cultures making laboratory detection and isolation of the virus extremely difficult. Thus, for many years, the only source of antigen consisted of sera from viremic patients. Recombinant antigens have been produced for use in serological assays in an attempt to circumvent these problems. See, e.g., Sisk and Berman, *Biotechnology* (1987) 5:1077-1080; U.S. Patent No. 6,204,044. Immunoenzymatic IgM capture assays have been used to detect anti-B19 IgM, as well as to diagnose recent B19 infection. The diagnostic performance of a number of commercially available tests, however, is not homogenous. In addition, IgM-based diagnostic tests cannot detect the virus during

the viremic stage of infection and once IgM antibodies are synthesized, they can remain in circulation for several months after the end of viremia.

The high prevalence of B19 antibodies in the normal population together with the fact that high viremia usually persists for only one week, make the use of serological based tests impractical. In addition, in immunocompromised patients, serological diagnosis may be unreliable.

Nucleic acid-based hybridization assays, such as dot blot and *in situ* hybridization have been used for B19 detection. These assays generally have detection limits of 1 to 0.1 pg viral DNA (~10^4-10^5 viral particles). PCR has greater sensitivity (~100 genome copies). However, DNA hybridization techniques are time consuming and limited in use and PCR is impractical for screening large numbers of samples.

Therefore, there remains a need for the development of reliable diagnostic tests to detect parvovirus B19 in viremic samples, in order to prevent transmission of the virus through blood and plasma derivatives or by close personal contact.

Summary of the Invention

The present invention is based on the discovery of unique primers and probes for use in nucleic acid-based assays, as well as on the development of a sensitive, reliable nucleic acid-based diagnostic test for the detection of parvovirus B19 DNA in biological samples from potentially infected individuals. The techniques described herein utilize extracted sample DNA as a template for amplification of conserved genomic regions of the B19 sequence using transcription-mediated amplification (TMA), as well as in a 5' nuclease assay, such as the TaqMan™ technique. The methods allow for the detection of B19 DNA in viremic samples having viral titers as low as 10^3 virus particles/ml. Accordingly, infected samples can be identified and excluded from transfusion, as well as from the preparation of blood derivatives. The probes and primers described herein are also useful in, for example, standard hybridization methods, as well as in PCR-based techniques, nucleic acid sequence-based amplification (NASBA) and in assays that utilize branched DNA molecules.

Accordingly, in one embodiment, the subject invention is directed to a method of detecting human parvovirus B19 infection in a biological sample. The method comprises:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein the nucleic acid comprises an RNA target sequence;

(b) reacting the isolated parvovirus B19 nucleic acid with a first oligonucleotide which comprises a first primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the RNA target sequence to complex therewith, wherein the first primer further comprises a promoter for a DNA-dependent RNA polymerase 5' and operably linked to the complexing sequence, wherein the reacting is done under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(c) extending the first primer in an extension reaction using the RNA target sequence as a template to give a first DNA primer extension product complementary to the RNA target sequence;

(d) separating the first DNA primer extension product from the RNA target sequence using an enzyme which selectively degrades the RNA target sequence;

(e) treating the DNA primer extension product with a second oligonucleotide which comprises a second primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the DNA primer extension product to complex therewith under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(f) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product, thereby producing a template for the DNA-dependent RNA polymerase;

(g) using the template to produce multiple RNA copies of the target sequence using a DNA-dependent RNA polymerase which recognizes the promoter sequence;

and (h) using the RNA copies of step (g), autocatalytically repeating steps (b) to (g)

to amplify the target sequence.

In certain embodiments, the method further comprises the steps of:

(i) adding a labeled oligonucleotide probe to the product of step (h), wherein the oligonucleotide probe is complementary to a portion of the target sequence, under conditions that provide for the hybridization of the probe with the target sequence to form a probe:target complex; and

(j) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

In additional embodiments, the label is an acridinium ester.

In yet further embodiments, the first and second primers, and the probe used in the methods above are derived from the VP1 region of the human parvovirus B19 genome, such as from the polynucleotide sequence depicted in any one of Figures 2A-2U or 11A-11Z.

In another embodiment, the invention is directed to a method of detecting human parvovirus B19 infection in a biological sample. The method comprises:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein the nucleic acid comprises an RNA target sequence;

(b) reacting the isolated parvovirus B19 nucleic acid with a first oligonucleotide which comprises a first primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the RNA target sequence to complex therewith, wherein the first primer further comprises a promoter for a DNA-dependent RNA polymerase 5' and operably linked to the complexing sequence, wherein the first primer comprises a sequence derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z and the reacting is done under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(c) extending the first primer in an extension reaction using the RNA target sequence as a template to give a first DNA primer extension product complementary to the RNA target sequence;

(d) separating the first DNA primer extension product from the RNA target sequence using an enzyme which selectively degrades the RNA target sequence;

(e) treating the DNA primer extension product with a second oligonucleotide which comprises a second primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the DNA primer extension product to complex therewith, wherein the second primer is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z and the treating is done under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(f) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product, thereby producing a template for the DNA-dependent RNA polymerase;

(g) using the template to produce multiple RNA copies of the target sequence using a DNA-dependent RNA polymerase which recognizes the promoter sequence;

and (h) using the RNA copies of step (g), autocatalytically repeating steps (b) to (g)

to amplify the target sequence;

(i) adding an acridinium ester-labeled oligonucleotide probe to the product of step (h), wherein the oligonucleotide probe is complementary to a portion of said target sequence and the probe is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U, wherein the probe is added under conditions that provide for the hybridization of the probe with the target sequence to form a probe:target complex; and

(j) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

In yet another embodiment, the invention is directed to a method for amplifying a target parvovirus B19 nucleotide sequence. The method comprises:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein the nucleic acid comprises an RNA target sequence;

(b) adding one or more primers capable of hybridizing to the RNA target sequence, wherein the one or more primers are derived from the polynucleotide sequences depicted in any one of Figures 2A-2U and Figures 11A-11Z;

(c) adding an oligonucleotide probe capable of hybridizing to the RNA target sequence 3' relative to the one or more primers;

(d) extending the one or more primers using a polymerase.

In certain embodiments, the RNA target sequence of step (a) is reverse transcribed to provide cDNA and the method can further comprise amplifying the cDNA using polymerase chain reaction (RT-PCR) or asymmetric gap ligase chain reaction (RT-AGLCR). In other embodiments, the polymerase is a thermostable polymerase, such as but not limited to Taq polymerase or Vent polymerase. In additional embodiments, the polymerase is *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, or T4 DNA polymerase.

In certain embodiments of the various methods described above, an internal control is provided. The internal control can be derived from the sequence of Figure 12 (SEQ ID NO:92). In additional embodiments, the internal control comprises SEQ ID NO:90.

In additional embodiments, the invention is directed to a method for detecting human parvovirus B19 infection in a biological sample. The method comprises:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein the nucleic acid comprises a target sequence;

(b) reacting the isolated parvovirus B19 nucleic acid with a detectably labeled probe sufficiently complementary to and capable of hybridizing with the target sequence, wherein the probe is derived from the polynucleotide sequences depicted in any one of Figures 2A-2U and Figures 11A-11Z, and further wherein the reacting is done under conditions that provide for the formation of a probe/target sequence complex; and

(c) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

In further embodiments, the invention is directed to a polynucleotide comprising a nucleotide sequence comprising any one of the nucleotide sequences depicted in Figures 2A-2U or Figures 11A-11Z.

In additional embodiments, the invention is directed to a polynucleotide, as above, wherein the nucleotide sequence consists of the nucleotide sequence depicted in Figures 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L, 2M, 2N, 2O, 2P, 2Q, 2R, 2S, 2T, 2U, 11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H, 11I, 11J, 11K, 11L, 11M, 11N, 11O, 11P, 11Q, 11R, 11S, 11T, 11U, 11V, 11W, 11X, 11Y or 11Z.

In still further embodiments, the subject invention is directed to a polynucleotide comprising a nucleotide sequence comprising any one of the nucleotide sequences depicted in Figures 3A-3C or 4A-4C.

In additional embodiments, the invention is directed to a polynucleotide as above, wherein the nucleotide sequence consists of the nucleotide sequence depicted in Figures 3A-3C or in Figures 4A-4C.

In another embodiment, the invention is directed to an oligonucleotide primer consisting of a promoter region recognized by a DNA-dependent RNA polymerase operably linked to a human parvovirus B19-specific complexing sequence of about 10 to about 75 nucleotides. In certain embodiments, the promoter region is the T7 promoter and said polymerase is T7 RNA polymerase. Additionally, the human parvovirus B19-specific sequence may be from the VP1 region of the human parvovirus B19 genome, such as from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z.

In yet further embodiments, the invention is directed an oligonucleotide primer consisting of a T7 promoter operably linked to a human parvovirus B19-specific complexing sequence of about 10 to about 75 nucleotides, wherein the human parvovirus B19-specific complexing sequence is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or 11A-11Z.

In another embodiment, the invention is directed to an oligonucleotide probe comprising a parvovirus B19-specific hybridizing sequence of about 10 to about 50 nucleotides linked to an acridinium ester label. In certain embodiments, the human

parvovirus B19-specific hybridizing sequence is from the VP1 region of the human parvovirus B19 genome, such as from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z.

In yet an additional embodiment, the invention is directed to a diagnostic test kit comprising one or more oligonucleotide primers described herein, and instructions for conducting the diagnostic test. In certain embodiments, the test kit further comprises an oligonucleotide probe comprising a parvovirus B19-specific hybridizing sequence of about 10 to about 50 nucleotides linked to an acridinium ester label.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

Brief Description of the Figures

Figure 1 is a diagrammatic representation of the human parvovirus B19 genome, depicting the various coding regions of the virus. Three PCR fragments are depicted, one with approximately 700 bp, corresponding to nucleotide positions 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936; one with approximately 370 bp within the 700 bp fragment, corresponding to nucleotide positions 3073-3442 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936; and one with approximately 214 bp corresponding to nucleotide positions 4728-4941 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936.

Figures 2A through 2U (SEQ ID NOS:1-21) depict DNA sequences from various parvovirus B19 isolates which include sequences corresponding to nucleotide positions 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936 (the 700 bp fragment from Figure 1). Figure 2A (SEQ ID NO:1) is the corresponding sequence from isolate CH47-26; Figure 2B (SEQ ID NO:2) is the corresponding sequence from isolate CH48-29; Figure 2C (SEQ ID NO:3) is the corresponding sequence from isolate CH33-2; Figure 2D (SEQ ID NO:4) is the corresponding sequence from isolate CH33-3; Figure 2E (SEQ ID NO:5) is the corresponding sequence from isolate CH33-4; Figure 2F (SEQ ID NO:6) is the

corresponding sequence from isolate CH42-7; Figure 2G (SEQ ID NO:7) is the corresponding sequence from isolate CH42-18; Figure 2H (SEQ ID NO:8) is the corresponding sequence from isolate CH42-19; Figure 2I (SEQ ID NO:9) is the corresponding sequence from isolate CH46-23; Figure 2J (SEQ ID NO:10) is the corresponding sequence from isolate CH1-1; Figure 2K (SEQ ID NO:11) is the corresponding sequence from isolate CH1-6; Figure 2L (SEQ ID NO:12) is the corresponding sequence from isolate CH2-8; Figure 2M (SEQ ID NO:13) is the corresponding sequence from isolate CH2-10; Figure 2N (SEQ ID NO:14) is the corresponding sequence from isolate CH2-11C; Figure 2O (SEQ ID NO:15) is the corresponding sequence from isolate CH5-13; Figure 2P (SEQ ID NO:16) is the corresponding sequence from isolate CH7-22; Figure 2Q (SEQ ID NO:17) is the corresponding sequence from isolate CH13-27; Figure 2R (SEQ ID NO:18) is the corresponding sequence from isolate CH14-33; Figure 2S (SEQ ID NO:19) is the corresponding sequence from isolate CH62-2; Figure 2T (SEQ ID NO:20) is the corresponding sequence from isolate CH64-2; and Figure 2U (SEQ ID NO:21) is the corresponding sequence from isolate CH67-2.

Figures 3A-3C (SEQ ID NO:22) show a sequence for the approximately 4.7 kbp PCR fragment shown in Figure 1 from parvovirus B19 clone 2-B1. The sequence is a 4677 nucleotide fragment corresponding to nucleotide positions 217-4893 of Shade et al., *J. Virol.* (1986) 58:921-936. The sequence depicted contains the parvovirus B19 full-length open reading frame which encodes NS1, VP1 and VP2, plus additional 5' and 3' untranslated sequences.

Figures 4A-4C (SEQ ID NO:23) show a sequence for the approximately 4.7 kbp PCR fragment shown in Figure 1 from parvovirus B19 clone 2-B6. The sequence is a 4677 nucleotide fragment corresponding to nucleotide positions 217-4893 of Shade et al., *J. Virol.* (1986) 58:921-936. The sequence depicted contains the parvovirus B19 full-length open reading frame which encodes NS1, VP1 and VP2, plus additional 5' and 3' untranslated sequences.

Figures 5A (SEQ ID NO:24) and 5B (SEQ ID NO:25) show the NS1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B1.

Figures 6A (SEQ ID NO:26) and 6B (SEQ ID NO:27) show the VP1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B1.

Figures 7A (SEQ ID NO:28) and 7B (SEQ ID NO:29) show the VP2 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B1.

Figures 8A (SEQ ID NO:30) and 8B (SEQ ID NO:31) show the NS1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B6.

Figures 9A (SEQ ID NO:32) and 9B (SEQ ID NO:33) show the VP1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B6.

Figures 10A (SEQ ID NO:34) and 10B (SEQ ID NO:35) show the VP2 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B6.

Figures 11A through 11Z (SEQ ID NOS:62-87) depict DNA sequences from various parvovirus B19 isolates which include sequences corresponding to nucleotide positions 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936 (the 700 bp fragment from Figure 1). Figure 11A (SEQ ID NO:62) is the corresponding sequence from isolate CH80-1; Figure 11B (SEQ ID NO:63) is the corresponding sequence from isolate CH81-3; Figure 11C (SEQ ID NO:64) is the corresponding sequence from isolate B19SCL1-4; Figure 11D (SEQ ID NO:65) is the corresponding sequence from isolate B19SCL2-1; Figure 11E (SEQ ID NO:66) is the corresponding sequence from isolate B19SCL3-1; Figure 11F (SEQ ID NO:67) is the corresponding sequence from isolate B19SCL4-3; Figure 11G (SEQ ID NO:68) is the corresponding sequence from isolate B19SCL5-2; Figure 11H (SEQ ID NO:69) is the corresponding sequence from isolate B19SCL6-2; Figure 11I (SEQ ID NO:70) is the corresponding sequence from isolate B19SCL7-3; Figure 11J (SEQ ID NO:71) is the corresponding sequence from isolate B19SCL8-2; Figure 11K (SEQ ID NO:72) is the corresponding sequence from isolate B19SCL9-1; Figure 11L (SEQ ID NO:73) is the corresponding sequence from isolate B19SCL9-9; Figure 11M (SEQ ID NO:74) is the corresponding sequence from isolate B19SCL10-2; Figure 11N (SEQ ID NO:75) is the corresponding sequence from isolate B19SCL11-1; Figure 11O (SEQ ID NO:76) is the corresponding sequence from isolate B19SCL12-1; Figure 11P (SEQ ID NO:77) is the corresponding sequence from isolate B19SCL13-3;

Figure 11Q (SEQ ID NO:78) is the corresponding sequence from isolate B19SCL14-1; Figure 11R (SEQ ID NO:79) is the corresponding sequence from isolate B19SCL15-3; Figure 11S (SEQ ID NO:80) is the corresponding sequence from isolate B19SCL16-2; Figure 11T (SEQ ID NO:81) is the corresponding sequence from isolate B19SCL17-1; Figure 11U (SEQ ID NO:82) is the corresponding sequence from isolate B19SCL18-1; Figure 11V (SEQ ID NO:83) is the corresponding sequence from isolate B19SCL19-1; Figure 11W (SEQ ID NO:84) is the corresponding sequence from isolate B19SCL20-3; Figure 11X (SEQ ID NO:85) is the corresponding sequence from isolate B19SCL21-3; Figure 11Y (SEQ ID NO:86) is the corresponding sequence from isolate B19SCL22-11; Figure 11Z (SEQ ID NO:87) is the corresponding sequence from isolate B19SCL2-14.

Figure 12 (SEQ ID NO:92) depicts an exemplary sequence from which an internal control (IC) can be derived for target capture and amplification.

Detailed Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, recombinant DNA techniques and virology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Fundamental Virology*, 2nd Edition, vol. I & II (B.N. Fields and D.M. Knipe, eds.); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *A Practical Guide to Molecular Cloning* (1984).

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more antigens, and the like.

The following amino acid abbreviations are used throughout the text:

Alanine: Ala (A)	Arginine: Arg (R)
Asparagine: Asn (N)	Aspartic acid: Asp (D)
Cysteine: Cys (C)	Glutamine: Gln (Q)
Glutamic acid: Glu (E)	Glycine: Gly (G)
Histidine: His (H)	Isoleucine: Ile (I)
Leucine: Leu (L)	Lysine: Lys (K)
Methionine: Met (M)	Phenylalanine: Phe (F)
Proline: Pro (P)	Serine: Ser (S)
Threonine: Thr (T)	Tryptophan: Trp (W)
Tyrosine: Tyr (Y)	Valine: Val (V)

I. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

The terms "polypeptide" and "protein" refer to a polymer of amino acid residues and are not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include postexpression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

A parvovirus B19 polypeptide is a polypeptide, as defined above, derived from a protein encoded by the B19 genome, such as from the nonstructural proteins, NS1 and NS2, as well as from the proteins which form the viral capsid, VP1

(approximately 781 amino acids in length) or VP2 (approximately 554 amino acids in length). Representative NS1, VP1 and VP2 sequences are depicted in Figures 5-10 herein. The polypeptide need not be physically derived from parvovirus B19, but may be synthetically or recombinantly produced. Moreover, the polypeptide may be derived from any of the various parvovirus B19 strains and isolates. A number of conserved and variable regions are known between these strains and isolates and, in general, the amino acid sequences of, for example, epitopes derived from these regions will have a high degree of sequence homology, e.g., amino acid sequence homology of more than 30%, preferably more than 40%, when the two sequences are aligned. Thus, for example, the term "VP1" polypeptide refers to native VP1 from any of the various parvovirus B19 strains and isolates. The complete genotypes and sequences for the above proteins of many parvovirus B19 strains and isolates are known. See, e.g., Shade et al., *J. Virol.* (1986) 58:921-936; Gallinella et al., *J. Virol. Methods* (1993) 41:203-211. Moreover, epitopes from parvovirus B19 derived from these regions are also known. See, e.g., U.S. Patent No. 5,436,127; and International Publication No. WO 91/12269.

The terms "analog" and "mutein" refer to biologically active derivatives of the reference molecule, or fragments of such derivatives, that retain desired activity, such as immunoreactivity in diagnostic assays. In general, the term "analog" refers to compounds having a native polypeptide sequence and structure with one or more amino acid additions, substitutions (generally conservative in nature) and/or deletions, relative to the native molecule, so long as the modifications do not destroy immunogenic activity. The term "mutein" refers to peptides having one or more peptide mimics ("peptoids"), such as those described in International Publication No. WO 91/04282. Preferably, the analog or mutein has at least the same immunoactivity as the native molecule. Methods for making polypeptide analogs and muteins are known in the art and are described further below.

Particularly preferred analogs include substitutions that are conservative in nature, i.e., those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four

families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cysteine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 conservative or non-conservative amino acid substitutions, or any integer between 5-25, so long as the desired function of the molecule remains intact. One of skill in the art may readily determine regions of the molecule of interest that can tolerate change by reference to Hopp/Woods and Kyte-Doolittle plots, well known in the art.

By "isolated" is meant, when referring to a polypeptide, that the indicated molecule is separate and discrete from the whole organism with which the molecule is found in nature or is present in the substantial absence of other biological macro-molecules of the same type. The term "isolated" with respect to a polynucleotide is a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences in association therewith; or a molecule disassociated from the chromosome.

A polynucleotide "derived from" or "specific for" a designated sequence refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the designated nucleotide sequence. The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest, but may be generated in any manner, including, but not limited to, chemical synthesis, replication,

reverse transcription or transcription, which is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either a sense or an antisense orientation of the original polynucleotide.

"Homology" refers to the percent similarity between two polynucleotide or two polypeptide moieties. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 50% , preferably at least about 75%, more preferably at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence similarity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100.

Readily available computer programs can be used to aid in the analysis of homology and identity, such as ALIGN, Dayhoff, M.O. in *Atlas of Protein Sequence and Structure* M.O. Dayhoff ed., 5 Suppl. 3:353-358, National biomedical Research Foundation, Washington, DC, which adapts the local homology algorithm of Smith and Waterman *Advances in Appl. Math.* 2:482-489, 1981 for peptide analysis. Programs for determining nucleotide sequence homology are available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI) for example, the BESTFIT, FASTA and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example, percent homology of a particular nucleotide sequence to a reference sequence can be

determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

Another method of establishing percent homology in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence homology." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: http://www.ncbi.nlm.gov/cgi-bin/BLAST.

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., *supra*; *DNA Cloning*, *supra*; *Nucleic Acid Hybridization*, *supra*.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their desired function. Thus, a given promoter operably linked to a nucleic acid sequence is capable of effecting the transcription, and in the case of a coding sequence, the expression of the coding

sequence when the proper transcription factors, etc., are present. The promoter need not be contiguous with the nucleic acid sequence, so long as it functions to direct the transcription and/or expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between the promoter sequence and the coding sequence, as can transcribed introns, and the promoter sequence can still be considered "operably linked" to the coding sequence.

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, viral, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation is not associated with all or a portion of the polynucleotide with which it is associated in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. In general, the gene of interest is cloned and then expressed in transformed organisms, as described further below. The host organism expresses the foreign gene to produce the protein under expression conditions.

A "control element" refers to a polynucleotide sequence which aids in the transcription and/or translation of a nucleotide sequence to which it is linked. The term includes promoters, transcription termination sequences, upstream regulatory domains, polyadenylation signals, untranslated regions, including 5'-UTRs and 3'-UTRs and when appropriate, leader sequences and enhancers, which collectively provide for the transcription and translation of a coding sequence in a host cell.

A "promoter" as used herein is a regulatory region capable of binding a polymerase and initiating transcription of a downstream (3' direction) nucleotide sequence operably linked thereto. For purposes of the present invention, a promoter sequence includes the minimum number of bases or elements necessary to initiate transcription of a sequence of interest at levels detectable above background. Within the promoter sequence is a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA or DNA polymerase. For example, promoter may be a nucleic acid sequence that is recognized by a DNA-dependent RNA polymerase ("transcriptase") as a signal to bind to the nucleic acid and begin the transcription of RNA at a specific site. For

binding, such transcriptases generally require DNA which is double-stranded in the portion comprising the promoter sequence and its complement; the template portion (sequence to be transcribed) need not be double-stranded. Individual DNA-dependent RNA polymerases recognize a variety of different promoter sequences which can vary markedly in their efficiency in promoting transcription. When an RNA polymerase binds to a promoter sequence to initiate transcription, that promoter sequence is not part of the sequence transcribed. Thus, the RNA transcripts produced thereby will not include that sequence.

A control sequence "directs the transcription" of a nucleotide sequence when RNA or DNA polymerase will bind the promoter sequence and transcribe the adjacent sequence.

A "DNA-dependent DNA polymerase" is an enzyme that synthesizes a complementary DNA copy from a DNA template. Examples are DNA polymerase I from *E. coli* and bacteriophage T7 DNA polymerase. All known DNA-dependent DNA polymerases require a complementary primer to initiate synthesis. Under suitable conditions, a DNA-dependent DNA polymerase may synthesize a complementary DNA copy from an RNA template.

A "DNA-dependent RNA polymerase" or a "transcriptase" is an enzyme that synthesizes multiple RNA copies from a double-stranded or partially-double stranded DNA molecule having a (usually double-stranded) promoter sequence. The RNA molecules ("transcripts") are synthesized in the 5' to 3' direction beginning at a specific position just downstream of the promoter. Examples of transcriptases are the DNA-dependent RNA polymerase from *E. coli* and bacteriophages T7, T3, and SP6.

An "RNA-dependent DNA polymerase" or "reverse transcriptase" is an enzyme that synthesizes a complementary DNA copy from an RNA template. All known reverse transcriptases also have the ability to make a complementary DNA copy from a DNA template; thus, they are both RNA- and DNA-dependent DNA polymerases. A primer is required to initiate synthesis with both RNA and DNA templates.

"RNAse H" is an enzyme that degrades the RNA portion of an RNA:DNA duplex. These enzymes may be endonucleases or exonucleases. Most reverse transcriptase enzymes normally contain an RNAse H activity in addition to their polymerase activity. However, other sources of the RNAse H are available without an associated polymerase activity. The degradation may result in separation of RNA from a RNA:DNA complex. Alternatively, the RNAse H may simply cut the RNA at various locations such that portions of the RNA melt off or permit enzymes to unwind portions of the RNA.

The terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule" are used herein to include a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes triple-, double- and single-stranded DNA, as well as triple-, double- and single-stranded RNA. It also includes modifications, such as by methylation and/or by capping, and unmodified forms of the polynucleotide. More particularly, the terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule" include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), any other type of polynucleotide which is an N– or C-glycoside of a purine or pyrimidine base, and other polymers containing nonnucleotidic backbones, for example, polyamide (e.g., peptide nucleic acids (PNAs)) and polymorpholino (commercially available from the Anti-Virals, Inc., Corvallis, Oregon, as Neugene) polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA. There is no intended distinction in length between the terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule," and these terms will be used interchangeably. These terms refer only to the primary structure of the molecule. Thus, these terms include, for example, 3'-deoxy-2',5'-DNA, oligodeoxyribonucleotide N3' P5' phosphoramidates, 2'-O-alkyl-substituted RNA, double- and single-stranded DNA, as well as double- and single-stranded RNA, DNA:RNA hybrids, and hybrids between PNAs and DNA or RNA,

and also include known types of modifications, for example, labels which are known in the art, methylation, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), with negatively charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), and with positively charged linkages (e.g., aminoalklyphosphoramidates, aminoalkylphosphotriesters), those containing pendant moieties, such as, for example, proteins (including nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide or oligonucleotide. In particular, DNA is deoxyribonucleic acid.

As used herein, the term "target nucleic acid region" or "target nucleic acid" denotes a nucleic acid molecule with a "target sequence" to be amplified. The target nucleic acid may be either single-stranded or double-stranded and may include other sequences besides the target sequence, which may not be amplified. The term "target sequence" refers to the particular nucleotide sequence of the target nucleic acid which is to be amplified. The target sequence may include a probe-hybridizing region contained within the target molecule with which a probe will form a stable hybrid under desired conditions. The "target sequence" may also include the complexing sequences to which the oligonucleotide primers complex and be extended using the target sequence as a template. Where the target nucleic acid is originally single-stranded, the term "target sequence" also refers to the sequence complementary to the "target sequence" as present in the target nucleic acid. If the "target nucleic acid" is originally double-stranded, the term "target sequence" refers to both the plus (+) and minus (-) strands.

The term "primer" or "oligonucleotide primer" as used herein, refers to an oligonucleotide which acts to initiate synthesis of a complementary DNA strand when placed under conditions in which synthesis of a primer extension product is induced,

i.e., in the presence of nucleotides and a polymerization-inducing agent such as a DNA or RNA polymerase and at suitable temperature, pH, metal concentration, and salt concentration. The primer is preferably single-stranded for maximum efficiency in amplification, but may alternatively be double-stranded. If double-stranded, the primer is first treated to separate its strands before being used to prepare extension products. This denaturation step is typically effected by heat, but may alternatively be carried out using alkali, followed by neutralization. Thus, a "primer" is complementary to a template, and complexes by hydrogen bonding or hybridization with the template to give a primer/template complex for initiation of synthesis by a polymerase, which is extended by the addition of covalently bonded bases linked at its 3' end complementary to the template in the process of DNA synthesis.

As used herein, the term "probe" or "oligonucleotide probe" refers to a structure comprised of a polynucleotide, as defined above, that contains a nucleic acid sequence complementary to a nucleic acid sequence present in the target nucleic acid analyte. The polynucleotide regions of probes may be composed of DNA, and/or RNA, and/or synthetic nucleotide analogs. When an "oligonucleotide probe" is to be used in a 5' nuclease assay, such as the TaqMan™ technique, the probe will contain at least one fluorescer and at least one quencher which is digested by the 5' endonuclease activity of a polymerase used in the reaction in order to detect any amplified target oligonucleotide sequences. In this context, the oligonucleotide probe will have a sufficient number of phosphodiester linkages adjacent to its 5' end so that the 5' to 3' nuclease activity employed can efficiently degrade the bound probe to separate the fluorescers and quenchers. When an oligonucleotide probe is used in the TMA technique, it will be suitably labeled, as described below.

It will be appreciated that the hybridizing sequences need not have perfect complementarity to provide stable hybrids. In many situations, stable hybrids will form where fewer than about 10% of the bases are mismatches, ignoring loops of four or more nucleotides. Accordingly, as used herein the term "complementary" refers to an oligonucleotide that forms a stable duplex with its "complement" under assay conditions, generally where there is about 90% or greater homology.

The terms "hybridize" and "hybridization" refer to the formation of complexes between nucleotide sequences which are sufficiently complementary to form complexes via Watson-Crick base pairing. Where a primer "hybridizes" with target (template), such complexes (or hybrids) are sufficiently stable to serve the priming function required by, e.g., the DNA polymerase to initiate DNA synthesis.

As used herein, the term "binding pair" refers to first and second molecules that specifically bind to each other, such as complementary polynucleotide pairs capable of forming nucleic acid duplexes. "Specific binding" of the first member of the binding pair to the second member of the binding pair in a sample is evidenced by the binding of the first member to the second member, or vice versa, with greater affinity and specificity than to other components in the sample. The binding between the members of the binding pair is typically noncovalent. Unless the context clearly indicates otherwise, the terms "affinity molecule" and "target analyte" are used herein to refer to first and second members of a binding pair, respectively.

The terms "specific-binding molecule" and "affinity molecule" are used interchangeably herein and refer to a molecule that will selectively bind, through chemical or physical means to a detectable substance present in a sample. By "selectively bind" is meant that the molecule binds preferentially to the target of interest or binds with greater affinity to the target than to other molecules. For example, a DNA molecule will bind to a substantially complementary sequence and not to unrelated sequences.

The "melting temperature" or "Tm" of double-stranded DNA is defined as the temperature at which half of the helical structure of DNA is lost due to heating or other dissociation of the hydrogen bonding between base pairs, for example, by acid or alkali treatment, or the like. The T_m of a DNA molecule depends on its length and on its base composition. DNA molecules rich in GC base pairs have a higher T_m than those having an abundance of AT base pairs. Separated complementary strands of DNA spontaneously reassociate or anneal to form duplex DNA when the temperature is lowered below the T_m. The highest rate of nucleic acid hybridization occurs approximately 25 °C below the T_m. The T_m may be estimated using the following

relationship: $T_m = 69.3 + 0.41(GC)\%$ (Marmur et al. (1962) *J. Mol. Biol.* 5:109-118).

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, that commonly includes antibodies produced by the subject. Typical samples that include such antibodies are known in the art and include but not limited to, blood, plasma, serum, fecal matter, urine, bone marrow, bile, spinal fluid, lymph fluid, samples of the skin, secretions of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, organs, biopsies and also samples of *in vitro* cell culture constituents including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium, e.g., recombinant cells, and cell components.

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, chromophores, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (e.g., biotin, avidin, strepavidin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range.

II. Modes of Carrying out the Invention

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of compositions and methods similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

As noted above, the present invention is based on the discovery of novel primers and probes and diagnostic methods for accurately detecting parvovirus B19 infection in a biological sample. The methods rely on sensitive nucleic acid-based

detection techniques that allow identification of parvovirus B19 target nucleic acid sequences in samples containing small amounts of virus.

In particular, the inventors herein have characterized regions within the parvovirus B19 genome which are desirable targets for diagnostic tests. Primers and probes derived from these regions are extremely useful for detection of parvovirus B19 infection in biological samples.

Parvovirus B19 primers and probes described above are used in nucleic acid-based assays for the detection of human parvovirus B19 infection in biological samples.

In particular, primers and probes for use in these assays are preferably derived from the approximately 4.7 kb fragment of the parvovirus B19 genome corresponding to nucleotide positions 217-4678 of Shade et al., *J. Virol.* (1986) 58:921-936. The nucleotide sequences of this region from two different parvovirus B19 isolates are depicted in Figures 3A-3C and 4A-4C herein. As explained above, this fragment contains the NS1, VP1 and VP2 coding regions.

Particularly preferred primers and probes for use with the present assays are designed from highly conserved regions of the parvovirus B19 genome to allow detection of parvovirus B19 infection caused by a variety of isolates. As described herein, a highly conserved region of the parvovirus B19 genome is found within the 700 bp region spanning nucleotide positions 2936-3635, numbered relative to the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936. This region is found within the VP1 region of the genome. The sequence of this region from 21 different parvovirus B19 isolates is shown herein in Figures 2A-2U. The sequences from an additional 26 isolates are shown in Figures 11A-11Z herein. A comparison of the sequences shows that this region displays from about 98% to 99.5% sequence homology from isolate to isolate, making it a highly desirable target sequence. Also desirable for the design of primers and probes is the 370 bp region found within VP1 which spans approximately nucleotide positions 3073-3442, numbered relative to Shade et al., *J. Virol.* (1986) 58:921-936, as well as the 214 bp fragment depicted in Figure 1 which occurs within the 3' portion of the 4.7 kb

fragment and spans nucleotide positions 4728-4941, numbered relative to Shade et al., *J. Virol.* (1986) 58:921-936.

The 4.7 kbp, 700 bp and 370 bp regions are readily obtained from additional isolates using portions of the parvovirus B19 sequence found within these particular regions as primers in PCR reactions such as those described herein, as well as in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,889,818, and based on the sequences provided herein. Another method of obtaining nucleotide sequences with the desired sequences is by annealing complementary sets of overlapping synthetic oligonucleotides produced in a conventional, automated polynucleotide synthesizer, followed by ligation with an appropriate DNA ligase and amplification of the ligated nucleotide sequence via PCR. See, e.g., Jayaraman et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:4084-4088. Once the sequences have been prepared or isolated, they can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Suitable vectors include, but are not limited to, plasmids, phages, transposons, cosmids, chromosomes or viruses which are capable of replication when associated with the proper control elements.

Recombinant clones are readily identified by restriction enzyme analysis and polyacryamide or agarose gel electrophresis, using techniques well known in the art, and described in the examples below.

Primers and probes for use in the assays herein are derived from these sequences and are readily synthesized by standard techniques, e.g., solid phase synthesis via phosphoramidite chemistry, as disclosed in U.S. Patent Nos. 4,458,066 and 4,415,732; Beaucage et al. (1992) *Tetrahedron* 48:2223-2311; and Applied Biosystems User Bulletin No. 13 (1 April 1987). Other chemical synthesis methods include, for example, the phosphotriester method described by Narang et al., *Meth. Enzymol.* (1979) 68:90 and the phosphodiester method disclosed by Brown et al., *Meth. Enzymol.* (1979) 68:109. Poly(A) or poly(C), or other non-complementary nucleotide extensions may be incorporated into probes using these same methods. Hexaethylene oxide extensions may be coupled to probes by methods known in the

art. Cload et al. (1991) *J. Am. Chem. Soc.* 113:6324-6326; U.S. Patent No. 4,914,210 to Levenson et al.; Durand et al. (1990) *Nucleic Acids Res.* 18:6353-6359; and Horn et al. (1986) *Tet. Lett.* 27:4705-4708. Typically, the primer sequences are in the range of between 10-75 nucleotides in length, such as 15-60, 20-40 and so on, more typically in the range of between 18-40 nucleotides long, and any length between the stated ranges. The typical probe is in the range of between 10-50 nucleotides long, such as 15-40, 18-30, and so on, and any length between the stated ranges.

Moreover, the probes may be coupled to labels for detection. There are several means known for derivatizing oligonucleotides with reactive functionalities which permit the addition of a label. For example, several approaches are available for biotinylating probes so that radioactive, fluorescent, chemiluminescent, enzymatic, or electron dense labels can be attached via avidin. See, e.g., Broken et al., *Nucl. Acids Res.* (1978) 5:363-384 which discloses the use of ferritin-avidin-biotin labels; and Chollet et al. *Nucl. Acids Res.* (1985) 13:1529-1541 which discloses biotinylation of the 5' termini of oligonucleotides via an aminoalkylphosphoramide linker arm. Several methods are also available for synthesizing amino-derivatized oligonucleotides which are readily labeled by fluorescent or other types of compounds derivatized by amino-reactive groups, such as isothiocyanate, N-hydroxysuccinimide, or the like, see, e.g., Connolly (1987) *Nucl. Acids Res.* 15:3131-3139, Gibson et al. (1987) *Nucl. Acids Res.* 15:6455-6467 and U.S. Patent No. 4,605,735 to Miyoshi et al. Methods are also available for synthesizing sulfhydryl-derivatized oligonucleotides which can be reacted with thiol-specific labels, see, e.g., U.S. Patent No. 4,757,141 to Fung et al., Connolly et al. (1985) *Nucl. Acids Res.* 13:4485-4502 and Spoat et al. (1987) *Nucl. Acids Res.* 15:4837-4848. A comprehensive review of methodologies for labeling DNA fragments is provided in Matthews et al., *Anal. Biochem.* (1988) 169:1-25.

For example, probes may be fluorescently labeled by linking a fluorescent molecule to the non-ligating terminus of the probe. Guidance for selecting appropriate fluorescent labels can be found in Smith et al., *Meth. Enzymol.* (1987) 155:260-301; Karger et al., *Nucl. Acids Res.* (1991) 19:4955-4962; Haugland (1989)

Handbook of Fluorescent Probes and Research Chemicals (Molecular Probes, Inc., Eugene, OR). Preferred fluorescent labels include fluorescein and derivatives thereof, such as disclosed in U.S. Patent No. 4,318,846 and Lee et al., *Cytometry* (1989) 10:151-164, and 6-FAM, JOE, TAMRA, ROX, HEX-1, HEX-2, ZOE, TET-1 or NAN-2, and the like.

Additionally, probes can be labeled with an acridinium ester (AE) using the techniques described below. Current technologies allow the AE label to be placed at any location within the probe. See, e.g., Nelson et al. (1995) "Detection of Acridinium Esters by Chemiluminescence" in *Nonisotopic Probing, Blotting and Sequencing*, Kricka L.J.(ed) Academic Press, San Diego, CA; Nelson et al. (1994) "Application of the Hybridization Protection Assay (HPA) to PCR" in *The Polymerase Chain Reaction*, Mullis et al. (eds.) Birkhauser, Boston, MA; Weeks et al., *Clin. Chem.* (1983) 29:1474-1479; Berry et al., *Clin. Chem.* (1988) 34:2087-2090. An AE molecule can be directly attached to the probe using non-nucleotide-based linker arm chemistry that allows placement of the label at any location within the probe. See, e.g., U.S. Patent Nos. 5,585,481 and 5,185,439.

In certain embodiments, an internal control (IC) or an internal standard is added to serve as a control for target capture and amplification. Preferably, the IC includes a sequence that differs from the target sequence, is capable of hybridizing with the probe sequences used for separating the oligonucleotides specific for the organism from the sample, and is capable of amplification. The use of the IC permits the control of the separation process, the amplification process, and the detection system, and permits the monitoring of assay performance and quantification for the sample(s). A representative sequence from which the IC can be obtained is shown in Figure 12. The IC can be included at any suitable point, for example, in the lysis buffer. In one embodiment, the IC comprises M13 ssDNA containing a nucleotide sequence from a parvovirus B19 and a unique sequence that hybridizes with the probe, for example, comprising sequences from the VP1 region, where the target sequence is modified by substituting or deleting 5-20 bases or more, preferably 5-15 bases, such as 5, 10 or 15, bases or any number within these ranges. The substituted

or deleted bases preferably occur over the entire length of the target sequence such that only 2 or 3 consecutive sequences are replaced. Thus for example, if the target sequence is CTACTTGCTGCGGGAGAAAAACACCT (SEQ ID NO:91), then the sequence may be substituted with, for example, AGCTAGACCTGCATGTCACTG (SEQ ID NO:90) in the IC.

The solid support may additionally include probes specific to the internal standard (IC probe), thereby facilitating capture when using the IC probe. The IC probe can optionally be coupled with a detectable label that is different from the detectable label for the target sequence. In embodiments where the detectable label is a fluorophore, the IC can be quantified spectrophotometrically and by limit of detection studies. Typically, the copy number of the IC which does not interfere with the target detection is determined by titrating the IC with a fixed IU of target, preferably at the lower end, and a standard curve is generated by diluting a sample of internationally accepted IU. For parvovirus B19 quantitation, an eight member panel of 8000 IU - 125 IU can be used.

In another embodiment, an IC, as described herein, is combined with RNA isolated from the sample according to standard techniques known to those of skill in the art, and described herein. The RNA is then reverse-transcribed using a reverse transcriptase to provide copy DNA. The cDNA sequences can be optionally amplified (e.g., by PCR) using labeled primers. The amplification products are separated, typically by electrophoresis, and the amount of radioactivity (proportional to the amount of amplified product) is determined. The amount of mRNA in the sample is then calculated by comparison with the signal produced by the known standards.

The primers and probes described above may be used in polymerase chain reaction (PCR)-based techniques to detect parvovirus B19 infection in biological samples. PCR is a technique for amplifying a desired target nucleic acid sequence contained in a nucleic acid molecule or mixture of molecules. In PCR, a pair of primers is employed in excess to hybridize to the complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic

acid as a template. The extension products become target sequences themselves after dissociation from the original target strand. New primers are then hybridized and extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. The PCR method for amplifying target nucleic acid sequences in a sample is well known in the art and has been described in, e.g., Innis et al. (eds.) *PCR Protocols* (Academic Press, NY 1990); Taylor (1991) *Polymerase chain reaction: basic principles and automation,* in *PCR: A Practical Approach*, McPherson et al. (eds.) IRL Press, Oxford; Saiki et al. (1986) *Nature* 324:163; as well as in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,889,818.

In particular, PCR uses relatively short oligonucleotide primers which flank the target nucleotide sequence to be amplified, oriented such that their 3' ends face each other, each primer extending toward the other. The polynucleotide sample is extracted and denatured, preferably by heat, and hybridized with first and second primers which are present in molar excess. Polymerization is catalyzed in the presence of the four deoxyribonucleotide triphosphates (dNTPs -- dATP, dGTP, dCTP and dTTP) using a primer- and template-dependent polynucleotide polymerizing agent, such as any enzyme capable of producing primer extension products, for example, *E. coli* DNA polymerase I, Klenow fragment of DNA polymerase I, T4 DNA polymerase, thermostable DNA polymerases isolated from *Thermus aquaticus* (*Taq*), available from a variety of sources (for example, Perkin Elmer), *Thermus thermophilus* (United States Biochemicals), *Bacillus stereothermophilus* (Bio-Rad), or *Thermococcus litoralis* ("Vent" polymerase, New England Biolabs). This results in two "long products" which contain the respective primers at their 5' ends covalently linked to the newly synthesized complements of the original strands. The reaction mixture is then returned to polymerizing conditions, e.g., by lowering the temperature, inactivating a denaturing agent, or adding more polymerase, and a second cycle is initiated. The second cycle provides the two original strands, the two long products from the first cycle, two new long products replicated from the original strands, and two "short products" replicated from the long products. The short products have the sequence of the target sequence with a primer

at each end. On each additional cycle, an additional two long products are produced, and a number of short products equal to the number of long and short products remaining at the end of the previous cycle. Thus, the number of short products containing the target sequence grow exponentially with each cycle. Preferably, PCR is carried out with a commercially available thermal cycler, e.g., Perkin Elmer.

RNAs may be amplified by reverse transcribing the mRNA into cDNA, and then performing PCR (RT-PCR), as described above. Alternatively, a single enzyme may be used for both steps as described in U.S. Patent No. 5,322,770. mRNA may also be reverse transcribed into cDNA, followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by Marshall et al. (1994) *PCR Meth. App.* 4:80-84.

The fluorogenic 5' nuclease assay, known as the TaqMan™ assay (Perkin-Elmer), is a powerful and versatile PCR-based detection system for nucleic acid targets. Hence, primers and probes derived from regions of the parvovirus B19 genome described herein can be used in TaqMan™ analyses to detect the presence of infection in a biological sample. Analysis is performed in conjunction with thermal cycling by monitoring the generation of fluorescence signals. The assay system dispenses with the need for gel electrophoretic analysis, and has the capability to generate quantitative data allowing the determination of target copy numbers.

The fluorogenic 5' nuclease assay is conveniently performed using, for example, AmpliTaq Gold™ DNA polymerase, which has endogenous 5' nuclease activity, to digest an internal oligonucleotide probe labeled with both a fluorescent reporter dye and a quencher (see, Holland et al., *Proc. Natl. Acad.Sci. USA* (1991) 88:7276-7280; and Lee et al., *Nucl. Acids Res.* (1993) 21:3761-3766). Assay results are detected by measuring changes in fluorescence that occur during the amplification cycle as the fluorescent probe is digested, uncoupling the dye and quencher labels and causing an increase in the fluorescent signal that is proportional to the amplification of target DNA.

The amplification products can be detected in solution or using solid supports. In this method, the TaqMan™ probe is designed to hybridize to a target sequence

within the desired PCR product. The 5' end of the TaqMan™ probe contains a fluorescent reporter dye. The 3' end of the probe is blocked to prevent probe extension and contains a dye that will quench the fluorescence of the 5' fluorophore. During subsequent amplification, the 5' fluorescent label is cleaved off if a polymerase with 5' exonuclease activity is present in the reaction. Excision of the 5' fluorophore results in an increase in fluorescence which can be detected.

In particular, the oligonucleotide probe is constructed such that the probe exists in at least one single-stranded conformation when unhybridized where the quencher molecule is near enough to the reporter molecule to quench the fluorescence of the reporter molecule. The oligonucleotide probe also exists in at least one conformation when hybridized to a target polynucleotide such that the quencher molecule is not positioned close enough to the reporter molecule to quench the fluorescence of the reporter molecule. By adopting these hybridized and unhybridized conformations, the reporter molecule and quencher molecule on the probe exhibit different fluorescence signal intensities when the probe is hybridized and unhybridized. As a result, it is possible to determine whether the probe is hybridized or unhybridized based on a change in the fluorescence intensity of the reporter molecule, the quencher molecule, or a combination thereof. In addition, because the probe can be designed such that the quencher molecule quenches the reporter molecule when the probe is not hybridized, the probe can be designed such that the reporter molecule exhibits limited fluorescence unless the probe is either hybridized or digested.

Accordingly, the present invention relates to methods for amplifying a target parvovirus B19 nucleotide sequence using a nucleic acid polymerase having 5' to 3' nuclease activity, one or more primers capable of hybridizing to the target B19 sequence, and an oligonucleotide probe capable of hybridizing to the target B19 sequence 3' relative to the primer. During amplification, the polymerase digests the oligonucleotide probe when it is hybridized to the target sequence, thereby separating the reporter molecule from the quencher molecule. As the amplification is conducted, the fluorescence of the reporter molecule is monitored, with fluorescence

corresponding to the occurrence of nucleic acid amplification. The reporter molecule is preferably a fluorescein dye and the quencher molecule is preferably a rhodamine dye.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 10-75 nucleotides long, more typically in the range of 20-45. The typical probe is in the range of between 10-50 nucleotides long, more typically 15-40 nucleotides in length.

If a solid support is used, the oligonucleotide probe may be attached to the solid support in a variety of manners. For example, the probe may be attached to the solid support by attachment of the 3' or 5' terminal nucleotide of the probe to the solid support. More preferably, the probe is attached to the solid support by a linker which serves to distance the probe from the solid support. The linker is usually at least 15-30 atoms in length, more preferably at least 15-50 atoms in length. The required length of the linker will depend on the particular solid support used. For example, a six atom linker is generally sufficient when high cross-linked polystyrene is used as the solid support.

A wide variety of linkers are known in the art which may be used to attach the oligonucleotide probe to the solid support. The linker may be formed of any compound which does not significantly interfere with the hybridization of the target sequence to the probe attached to the solid support. The linker may be formed of a homopolymeric oligonucleotide which can be readily added on to the linker by automated synthesis. Alternatively, polymers such as functionalized polyethylene glycol can be used as the linker. Such polymers are preferred over homopolymeric oligonucleotides because they do not significantly interfere with the hybridization of probe to the target oligonucleotide. Polyethylene glycol is particularly preferred.

The linkages between the solid support, the linker and the probe are preferably not cleaved during removal of base protecting groups under basic conditions at high temperature. Examples of preferred linkages include carbamate and amide linkages.

Examples of preferred types of solid supports for immobilization of the oligonucleotide probe include controlled pore glass, glass plates, polystyrene, avidin-coated polystyrene beads, cellulose, nylon, acrylamide gel and activated dextran.

For a detailed description of the TaqMan™ assay, reagents and conditions for use therein, see, e.g., Holland et al., *Proc. Natl. Acad. Sci, U.S.A.* (1991) 88:7276-7280; U.S. Patent Nos. 5,538,848, 5,723,591, and 5,876,930.

The parvovirus B19 sequences described herein may also be used as a basis for transcription-mediated amplification (TMA) assays. TMA provides a method of identifying target nucleic acid sequences present in very small amounts in a biological sample. Such sequences may be difficult or impossible to detect using direct assay methods. In particular, TMA is an isothemal, autocatalytic nucleic acid target amplification system that can provide more than a billion RNA copies of a target sequence. The assay can be done qualitatively, to accurately detect the presence or absence of the target sequence in a biological sample. The assay can also provide a quantitative measure of the amount of target sequence over a concentration range of several orders of magnitude. TMA provides a method for autocatalytically synthesizing multiple copies of a target nucleic acid sequence without repetitive manipulation of reaction conditions such as temperature, ionic strength and pH.

Generally, TMA includes the following steps: (a) isolating nucleic acid, including RNA, from the biological sample of interest suspected of being infected with parvovirus B19; and (b) combining into a reaction mixture (i) the isolated nucleic acid, (ii) first and second oligonucleotide primers, the first primer having a complexing sequence sufficiently complementary to the 3' terminal portion of an RNA target sequence, if present (for example the (+) strand), to complex therewith, and the second primer having a complexing sequence sufficiently complementary to the 3' terminal portion of the target sequence of its complement (for example, the (-) strand) to complex therewith, wherein the first oligonucleotide further comprises a sequence 5' to the complexing sequence which includes a promoter, (iii) a reverse transcriptase or RNA and DNA dependent DNA polymerases, (iv) an enzyme activity which selectively degrades the RNA strand of an RNA-DNA complex (such as an

RNAse H) and (v) an RNA polymerase which recognizes the promoter.

The components of the reaction mixture may be combined stepwise or at once. The reaction mixture is incubated under conditions whereby an oligonucleotide/target sequence is formed, including DNA priming and nucleic acid synthesizing conditions (including ribonucleotide triphosphates and deoxyribonucleotide triphosphates) for a period of time sufficient to provide multiple copies of the target sequence. The reaction advantageously takes place under conditions suitable for maintaining the stability of reaction components such as the component enzymes and without requiring modification or manipulation of reaction conditions during the course of the amplification reaction. Accordingly, the reaction may take place under conditions that are substantially isothermal and include substantially constant ionic strength and pH. The reaction conveniently does not require a denaturation step to separate the RNA-DNA complex produced by the first DNA extension reaction.

Suitable DNA polymerases include reverse transcriptases, such as avian myeloblastosis virus (AMV) reverse transcriptase (available from, e.g., Seikagaku America, Inc.) and Moloney murine leukemia virus (MMLV) reverse transcriptase (available from, e.g., Bethesda Research Laboratories).

Promoters or promoter sequences suitable for incorporation in the primers are nucleic acid sequences (either naturally occurring, produced synthetically or a product of a restriction digest) that are specifically recognized by an RNA polymerase that recognizes and binds to that sequence and initiates the process of transcription whereby RNA transcripts are produced. The sequence may optionally include nucleotide bases extending beyond the actual recognition site for the RNA polymerase which may impart added stability or susceptibility to degradation processes or increased transcription efficiency. Examples of useful promoters include those which are recognized by certain bacteriophage polymerases such as those from bacteriophage T3, T7 or SP6, or a promoter from *E. coli*. These RNA polymerases are readily available from commercial sources, such as New England Biolabs and Epicentre.

Some of the reverse transcriptases suitable for use in the methods herein have

an RNAse H activity, such as AMV reverse transcriptase. It may, however, be preferable to add exogenous RNAse H, such as *E. coli* RNAse H, even when AMV reverse transcriptase is used. RNAse H is readily available from, e.g., Bethesda Research Laboratories.

The RNA transcripts produced by these methods may serve as templates to produce additional copies of the target sequence through the above-described mechanisms. The system is autocatalytic and amplification occurs autocatalytically without the need for repeatedly modifying or changing reaction conditions such as temperature, pH, ionic strength or the like.

Detection may be done using a wide variety of methods, including direct sequencing, hybridization with sequence-specific oligomers, gel electrophoresis and mass spectrometry. these methods can use heterogeneous or homogeneous formats, isotopic or nonisotopic labels, as well as no labels at all.

One preferable method of detection is the use of target sequence-specific oligonucleotide probes, derived from the 4.7 kbp, 700 bp, 370 bp and 214 bp fragments described above. The probes may be used in hybridization protection assays (HPA). In this embodiment, the probes are conveniently labeled with acridinium ester (AE), a highly chemiluminescent molecule. See, e.g., Nelson et al. (1995) "Detection of Acridinium Esters by Chemiluminescence" in *Nonisotopic Probing, Blotting and Sequencing*, Kricka L.J.(ed) Academic Press, San Diego, CA; Nelson et al. (1994) "Application of the Hybridization Protection Assay (HPA) to PCR" in *The Polymerase Chain Reaction*, Mullis et al. (eds.) Birkhauser, Boston, MA; Weeks et al., *Clin. Chem.* (1983) 29:1474-1479; Berry et al., *Clin. Chem.* (1988) 34:2087-2090. One AE molecule is directly attached to the probe using a non-nucleotide-based linker arm chemistry that allows placement of the label at any location within the probe. See, e.g., U.S. Patent Nos. 5,585,481 and 5,185,439. Chemiluminescence is triggered by reaction with alkaline hydrogen peroxide which yields an excited N-methyl acridone that subsequently collapses to ground state with the emission of a photon. Additionally, AE causes ester hydrolysis which yields the nonchemiluminescent –methyl acridinium carboxylic acid.

When the AE molecule is covalently attached to a nucleic acid probe, hydrolysis is rapid under mildly alkaline conditions. When the AE-labeled probe is exactly complementary to the target nucleic acid, the rate of AE hydrolysis is greatly reduced. Thus, hybridized and unhybridized AE-labeled probe can be detected directly in solution, without the need for physical separation.

HPA generally consists of the following steps: (a) the AE-labeled probe is hybridized with the target nucleic acid in solution for about 15 to about 30 minutes. A mild alkaline solution is then added and AE coupled to the unhybridized probe is hydrolyzed. This reaction takes approximately 5 to 10 minutes. The remaining hybrid-associated AE is detected as a measure of the amount of target present. This step takes approximately 2 to 5 seconds. Preferably, the differential hydrolysis step is conducted at the same temperature as the hybridization step, typically at 50 to 70 °C. Alternatively, a second differential hydrolysis step may be conducted at room temperature. This allows elevated pHs to be used, for example in the range of 10-11, which yields larger differences in the rate of hydrolysis between hybridized and unhybridized AE-labeled probe. HPA is described in detail in, e.g., U.S. Patent Nos. 6,004,745; 5,948,899; and 5,283,174.

TMA is described in detail in, e.g., U.S. Patent No. 5,399,491. In one example of a typical assay, an isolated nucleic acid sample, suspected of containing a parvovirus B19 target sequence, is mixed with a buffer concentrate containing the buffer, salts, magnesium, nucleotide triphosphates, primers, dithiothreitol, and spermidine. The reaction is optionally incubated at about 100 °C for approximately two minutes to denature any secondary structure. After cooling to room temperature, reverse transcriptase, RNA polymerase, and RNAse H are added and the mixture is incubated for two to four hours at 37 °C. The reaction can then be assayed by denaturing the product, adding a probe solution, incubating 20 minutes at 60 °C, adding a solution to selectively hydrolyze the unhybridized probe, incubating the reaction six minutes at 60 °C, and measuring the remaining chemiluminescence in a luminometer.

The oligonucleotide molecules of the present invention may also be used in

nucleic acid sequence-based amplification (NASBA). This method is a promoter-directed, enzymatic process that induces *in vitro* continuous, homogeneous and isothermal amplification of a specific nucleic acid to provide RNA copies of the nucleic acid. The reagents for conducting NASBA include a first DNA primer with a 5' tail comprising a promoter, a second DNA primer, reverse transcriptase, RNAse-H, T7 RNA polymerase, NTP's and dNTP's. Using NASBA, large amounts of single-stranded RNA are generated from either single-stranded RNA or DNA, or double-stranded DNA. When RNA is to be amplified, the ssRNA serves as a template for the synthesis of a first DNA strand by elongation of a first primer containing an RNA polymerase recognition site. This DNA strand in turn serves as the template for the synthesis of a second, complementary, DNA strand by elongation of a second primer, resulting in a double- stranded active RNA-polymerase promoter site, and the second DNA strand serves as a template for the synthesis of large amounts of the first template, the ssRNA, with the aid of a RNA polymerase. The NASBA technique is known in the art and described in, e.g., European Patent 329,822, International Patent Application No. WO 91/02814, and U.S. Patent Nos. 6,063,603, 5,554,517 and 5,409,818.

The parvovirus B19 sequences described herein are also useful in nucleic acid hybridization and amplification techniques that utilize branched DNA molecules. In a basic nucleic acid hybridization assay, single-stranded analyte nucleic acid is hybridized to a labeled single-stranded nucleic acid probe and resulting labeled duplexes are detected. Variations of this basic scheme have been developed to facilitate separation of the duplexes to be detected from extraneous materials and/or to amplify the signal that is detected. One method for amplifying the signal uses amplification multimers that are polynucleotides with a first segment that hybridizes specifically to the analyte nucleic acid or a strand of nucleic acid bound to the analyte and iterations of a second segment that hybridizes specifically to a labeled probe. The amplification is theoretically proportional to the number of iterations of the second segment. The multimers may be either linear or branched. Two general types of branched multimers are useful in these techniques: forked and combed. Methods for

making and using branched nucleic acid molecules are known in the art and described in, e.g., U.S. Patent No. 5,849,481.

In another aspect of the invention, two or more of the tests described above are performed to confirm the presence of the organism. For example, if the first test used the transcription mediated amplification (TMA) to amplify the nucleic acids for detection, then an alternative nucleic acid testing (NAT) assay is performed, for example, by using PCR amplification, RT PCR, and the like, as described herein. Thus, parvovirus B19 can be specifically and selectively detected even when the sample contains other organisms, such as HIV, and Hepatitis B virus, for example.

As is readily apparent, design of the assays described herein are subject to a great deal of variation, and many formats are known in the art. The above descriptions are merely provided as guidance and one of skill in the art can readily modify the described protocols, using techniques well known in the art.

The above-described assay reagents, including the primers, probes, solid support with bound probes, as well as other detection reagents, can be provided in kits, with suitable instructions and other necessary reagents, in order to conduct the assays as described above. The kit will normally contain in separate containers the combination of primers and probes (either already bound to a solid matrix or separate with reagents for binding them to the matrix), control formulations (positive and/or negative), labeled reagents when the assay format requires same and signal generating reagents (e.g., enzyme substrate) if the label does not generate a signal directly. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay usually will be included in the kit. The kit can also contain, depending on the particular assay used, other packaged reagents and materials (i.e. wash buffers and the like). Standard assays, such as those described above, can be conducted using these kits.

III. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

In the following examples, enzymes were purchased from commercial sources, and used according to the manufacturers' directions. Nitrocellulose filters and the like were also purchased from commercial sources.

In the isolation of DNA fragments, except where noted, all DNA manipulations were done according to standard procedures. See, Sambrook et al., *supra*. Restriction enzymes, T_4 DNA ligase, *E. coli*, DNA polymerase I, Klenow fragment, and other biological reagents can be purchased from commercial suppliers and used according to the manufacturers' directions. Double stranded DNA fragments were separated on agarose gels.

Example 1

Parvovirus B19 Nucleic Acid Extraction for PCR

Human serum samples that had previously tested positive for human parvovirus B19 by either IgM or PCR tests were obtained from commercial sources and used to isolate DNA for subsequent PCR experiments. Samples were stored at -80 °C until used.

DNA was extracted from 0.2 mL of serum using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) following the manufacturer's specifications with the following considerations. Carrier DNA was added to the lysis buffer to enhance nucleic acid binding and yield. In particular, an amount of 5.6 μg per sample of poly-adenylic acid 5' (Sigma, St. Louis, MO) or poly-dA (Roche, Indianapolis, IN) was added. Additionally, parvovirus B19 DNA was eluted with 200 μL of buffer AE (Qiagen) instead of water.

Example 2

Detection of Parvovirus B19 Nucleic Acid-Positive Samples by PCR

Two different PCR procedures were used to amplify parvovirus B19 fragments. One method, described in detail below, was used to amplify fragments of approximately 700 bp, 370 bp and 214 bp (see, Figure 1). High Fidelity Expand PCR (Roche) was used to amplify fragments of approximately 4.7 kb. The approximately 700 bp fragment corresponds to nucleotide positions 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936. The approximately 370 bp occurs within the 700 bp fragment at nucleotide positions 3073-3442. The approximately 4.7 kb fragment is a 4677 nucleotide fragment corresponding to nucleotide positions 217-4893 of Shade et al., *J. Virol.* (1986) 58:921-936.

In order to amplify the B19 fragments of approximately 700 bp, 370 bp and 214 bp, the primers shown in Table 1 were used.

Table 1

Primer	Sequence	PCR product	Genomic region
VP-5	AGGAAGTTTGCCGGAAGTTC (SEQ ID NO:36)	370 bp	VP1
VP-3	GTGCTGAAACTCTAAAGGTG (SEQ ID NO:37)	370bp	VP1
VP2-5	GACATGGATATGAAAAGCCTGAAG (SEQ ID NO:38)	214 bp	VP1/VP2
VP2-3	GTTGTTCATATCTGGTTAAGTACT (SEQ ID NO:39)	214 bp	VP1/VP2
K-1sp	ATAAATCCATATACTCATT (SEQ ID NO:40)	700 bp	VP1/VP2
K-2sp	CTAAAGTATCCTGACCTTG (SEQ ID NO:41)	700 bp	VP1/VP2

For this experiment, PCR was performed in a final volume of 100 μL using 2 μL of purified parvovirus B19 DNA (purified as described above), 0.2 mM of each deoxy nucleotide triphosphate and 1.25 units of Pfu DNA polymerase (Stratagene, La Jolla, CA). The amplification profile involved denaturation at 94 °C for 2 min., primer annealing at 37 °C for 3 min. and extension at 72 °C for 3 min. for 35 cycles. A 3-min. preincubation at 94 °C to ensure initial denaturation and a final 7-min. incubation at 72 °C to ensure the full extension of fragments preceded and followed, respectively, the 35 PCR cycles. PCR products were electrophoresed on 7% polyacrylamide gels, stained with ethidium bromide and visualized under an UV source. Purification of amplified fragments was carried out using the QiaQuick PCR purification kit (QIAGEN).

Nested PCR to amplify the 370 bp B19 fragment was performed when the 700 bp band was not visualized on the polyacrylamide gels. The 700 bp DNA material was used for the nested PCR using primers shown in Table 1.

High Fidelity Expand PCR (Roche) was used to amplify the parvovirus B19 fragment of 4.7 kb as follows. The High Fidelity Expand PCR kit (Roche) and primers Hicks-5 (5'CCCGCCTTATGCAAATGGGCAG3') (SEQ ID NO:42) and Hicks-3 (5'TTGTGTTAGGCTGTCTTATAGG3') (SEQ ID NO:43) were used following the vendor's recommendations. Amplification conditions were 94 °C for 1 min., 50 °C for 2 min. and 68 °C for 4 min. for 35 or 45 cycles. A pre-incubation at 94 °C for 2 min. and a post incubation at 75 °C for 7 min. were also included. The PCR products were separated on 1% agarose gels and purified using the PCR Purification kit (Promega, Madison, WI).

Example 3

Cloning of Parvovirus B19 DNA Fragments

The PCR fragments were cloned into TOPO-TA vectors (Invitrogen, Carlsbad, CA). Cloning into these vectors is highly facilitated when the amplified DNA contains a single deoxyadenosine (A) at its 3' end. Accordingly, a catalytic reaction to add the 3' (A) overhead was used. The reaction mix contained 1.25 mM of dATP, 0.5 units of Taq polymerase (Perkin Elmer, Boston, MA) and proceeded at 72 °C for 15 min.

PCR fragments were cloned into the pCR2.1-TOPO vector using Invitrogen's TA cloning kit (TOPO™ TA Cloning® Kit with One Shot TOP10 Electrocompetent Cells) following the manufacturer's specifications. Bacterial cells were incubated at 37 °C on Luria Broth plates containing ampicillin at 100 µg/ml, 0.66 mM IPTG and 0.033% X-Gal. A number of white colonies were inoculated in 4 mL of Luria-Broth ampicillin (100 µg/ml) and incubated overnight at 37 °C with shaking. Three mL of the overnight cultures were used to prepare plasmid DNA using the QIAprep Miniprep kit (QIAGEN). Recombinant clones were identified by restriction enzyme

analysis with *Eco*RI (New England and Biolabs) and 7% polyacryamide or 1% agarose gel electrophresis as described above.

In order to determine the DNA sequences of the clones, large amounts of plasmids from recombinant clones were prepared as above and the DNA suspended in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at 0.2 mg/ml. Nucleotide sequence determination of the parvovirus B19 fragments was performed using an Applied Biosystems Model 373 (or Model 377) DNA Sequencer system.

Figures 2A through 2U (SEQ ID NOS:1-21) depict DNA sequences from 21 parvovirus B19 isolates, purified, amplified and sequenced as described above, which correspond to nucleotide positions 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936 (the 700 bp fragment from Figure 1 and described above). Figure 2A (SEQ ID NO:1) is the sequence from isolate CH47-26; Figure 2B (SEQ ID NO:2) is the sequence from isolate CH48-29; Figure 2C (SEQ ID NO:3) is the sequence from isolate CH33-2; Figure 2D (SEQ ID NO:4) is the sequence from isolate CH33-3; Figure 2E (SEQ ID NO:5) is the sequence from isolate CH33-4; Figure 2F (SEQ ID NO:6) is the sequence from isolate CH42-7; Figure 2G (SEQ ID NO:7) is the sequence from isolate CH42-18; Figure 2H (SEQ ID NO:8) is the sequence from isolate CH42-19; Figure 2I (SEQ ID NO:9) is the sequence from isolate CH46-23; Figure 2J (SEQ ID NO:10) is the sequence from isolate CH1-1; Figure 2K (SEQ ID NO:11) is the sequence from isolate CH1-6; Figure 2L (SEQ ID NO:12) is the sequence from isolate CH2-8; Figure 2M (SEQ ID NO:13) is the sequence from isolate CH2-10; Figure 2N (SEQ ID NO:14) is the sequence from isolate CH2-11C; Figure 2O (SEQ ID NO:15) is the sequence from isolate CH5-13; Figure 2P (SEQ ID NO:16) is the sequence from isolate CH7-22; Figure 2Q (SEQ ID NO:17) is the sequence from isolate CH13-27; Figure 2R (SEQ ID NO:18) is the sequence from isolate CH14-33; Figure 2S (SEQ ID NO:19) is the sequence from isolate CH62-2; Figure 2T (SEQ ID NO:20) is the sequence from isolate CH64-2; and Figure 2U (SEQ ID NO:21) is the sequence from isolate CH67-2.

Figures 11A through 11Z (SEQ ID NOS:62-87) depict DNA sequences

from an additional 26 parvovirus B19 isolates, purified, amplified and sequenced as described above, which correspond to nucleotide positions 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936 (the 700 bp fragment from Figure 1 and described above). Figure 11A (SEQ ID NO:62) is the sequence from isolate CH80-1; Figure 11B (SEQ ID NO:63) is the sequence from isolate CH81-3; Figure 11C (SEQ ID NO:64) is the sequence from isolate B19SCL1-4; Figure 11D (SEQ ID NO:65) is the sequence from isolate B19SCL2-1; Figure 11E (SEQ ID NO:66) is the sequence from isolate B19SCL3-1; Figure 11F (SEQ ID NO:67) is the sequence from isolate B19SCL4-3; Figure 11G (SEQ ID NO:68) is the sequence from isolate B19SCL5-2; Figure 11H (SEQ ID NO:69) is the sequence from isolate B19SCL6-2; Figure 11I (SEQ ID NO:70) is the sequence from isolate B19SCL7-3; Figure 11J (SEQ ID NO:71) is the sequence from isolate B19SCL8-2; Figure 11K (SEQ ID NO:72) is the sequence from isolate B19SCL9-1; Figure 11L (SEQ ID NO:73) is the sequence from isolate B19SCL9-9; Figure 11M (SEQ ID NO:74) is the sequence from isolate B19SCL10-2; Figure 11N (SEQ ID NO:75) is the sequence from isolate B19SCL11-1; Figure 11O (SEQ ID NO:76) is the sequence from isolate B19SCL12-1; Figure 11P (SEQ ID NO:77) is the sequence from isolate B19SCL13-3; Figure 11Q (SEQ ID NO:78) is the sequence from isolate B19SCL14-1; Figure 11R (SEQ ID NO:79) is the sequence from isolate B19SCL15-3; Figure 11S (SEQ ID NO:80) is the sequence from isolate B19SCL16-2; Figure 11T (SEQ ID NO:81) is the sequence from isolate B19SCL17-1; Figure 11U (SEQ ID NO:82) is the sequence from isolate B19SCL18-1; Figure 11V (SEQ ID NO:83) is the sequence from isolate B19SCL19-1; Figure 11W (SEQ ID NO:84) is the sequence from isolate B19SCL20-3; Figure 11X (SEQ ID NO:85) is the sequence from isolate B19SCL21-3; Figure 11Y (SEQ ID NO:86) is the sequence from isolate B19SCL22-11; Figure 11Z (SEQ ID NO:87) is the sequence from isolate B19SCL2-14.

Sequence comparisons revealed approximately 98% to 99.5% sequence homology of this 700 bp sequence between the various isolates.

Figures 3A-3C (SEQ ID NO:22) show the sequence for the approximately 4.7 kbp PCR fragment shown in Figure 1 and described above from parvovirus B19 clone

2-B1. The sequence depicted in the figures is a 4677 nucleotide fragment corresponding to nucleotide positions 217-4893 of Shade et al., *J. Virol.* (1986) 58:921-936. The sequence depicted contains the parvovirus B19 full-length open reading frame which encodes NS1, VP1 and VP2, plus additional 5' and 3' untranslated sequences. The fragment sequenced contained an additional nucleotide in the 5' non-coding region between nucleotide position 367 and 368 of the B19 sequence reported by Shade et al., *J. Virol.* (1986) 58:921-936.

Figures 4A-4C (SEQ ID NO:23) show the sequence for the approximately 4.7 kbp PCR fragment shown in Figure 1 from parvovirus B19 clone 2-B6. The sequence is a 4677 nucleotide fragment corresponding to nucleotide positions 217-4893 of Shade et al., *J. Virol.* (1986) 58:921-936. The sequence depicted contains the parvovirus B19 full-length open reading frame which encodes NS1, VP1 and VP2, plus additional 5' and 3' untranslated sequences. The fragment sequenced contained an additional nucleotide in the 5' non-coding region between nucleotide position 367 and 368 of the B19 sequence reported by Shade et al., *J. Virol.* (1986) 58:921-936.

Example 4

Cloning and Expression of Parvovirus B19 NS1, VP1 and VP2 Recombinant Proteins.

Fragments encoding NS1, VP1 and VP2 (see Figure 1) were amplified using the 4.7 kb fragment of parvovirus B19 cloned in pCR2.1-TOPO (described above). In particular, PCR primers (see below) were designed to PCR out the NS1, VP1, and VP2 regions of parvovirus B19. To facilitate the cloning of these regions into yeast expression vectors, *Xba*I, *Hind*III and *Sal*I restriction sites were introduced in the primers as required.

The primers used to clone and amplify parvovirus B19 fragments for yeast expression of NS1, VP1 and VP2 recombinant proteins were based on the sequences obtained above and were as follows:

NS1-5 (sense primer)

5'ATACTCTCTAGACAAAACAAAATGGAGCTATTTAGAGGGGTGCTTCAAGTTTCT3'

(SEQ ID NO:44)

NS1-3 (anti-sense primer)
5' GAGTATGTCGACTTACTCATAATCTACAAAGCTTTGCAATCCAGACAG3' (SEQ ID NO:45)

VP1-5SN (sense primer)
5'ATACTCAAGCTTACAAAACAAAATGAGTAAAGAAAGTGGCAAATGGTGGGAAAGT3' (SEQ ID NO:46)

VPALL-3 (anti-sense primer)
5'GAGTATGTCGACTTACAATGGGTGCACACGGCTTTTGGCTGTCCACAATTC3' (SEQ ID NO:47)

VP2-5SN (sense primer)
5'ATACTCAAGCTTACAAAACAAAATGACTTCAGTTAATTCTGCAGAAGCCAGCACT3' (SEQ ID NO:48)

PCR primers were synthesized, purified and suspended in 300 μL of dH_20 and their optical densities at 260 nm determined. The reaction mix contained 0.25 ng of template, 100 pmol of each primer, 10μL of 1.25 mM of each dNTP and 1 unit of Taq polymerase (Perkin Elmer, Boston, MA) in a final volume of 50 μL. Amplification conditions were 94°C for 1 min., 50°C for 2 min. and 68°C for 4 min. for 35 cycles. A 7-min. post-incubation at 75°C was added to ensure the full extension of fragments. Aliquots of 5 μL were used to check PCR synthesis by electrophoresis on 1% agarose gels. The entire PCR product was then electrophoresed and fragments exhibiting the expected sizes were purified from the gels using the PCR Purification kit (Promega) following the vendor's recommendations. Approximately 0.8 μg of purified PCR DNA was digested with the appropriate restriction enzymes (Roche) for 3h at 37°C and the products were further purified using the Promega PCR Purification kit.

Plasmid pBS24.1 was used for heterologous expression of the parvovirus B19 recombinant proteins. This yeast expression vector contains 2μ sequences and inverted repeats (IR) for autonomous replication in yeast, the α-factor terminator to

ensure transcription termination, and the yeast *leu2-d* and URA3 for selection. The ColE1 origin of replication and the β-lactamase gene are also present for propagation and selection in *E. coli* (Pichuantes et al. (1996) "Expression of Heterologous Gene Products in Yeast." In: *Protein Engineering: A Guide to Design and Production*, Chapter 5. J. L. Cleland and C. Craik, eds., Wiley-Liss, Inc., New York, N.Y. pp. 129-161. Plasmid pBS24.1 was digested with *Bam*HI/*Sal*I and dephosphorylated with 10 units of calf intestine alkaline phosphatase (Boheringer Manheim, Indianapolis, IN) under the conditions recommended by the vendor. The digested and purified PCR fragments were mixed with *Bam*HI/*Sal*I digested pBS24.1 and with a DNA fragment containing the yeast hybrid promoter ADH2/GAPDH (Cousens et al., *Gene* (1987) 61:265- 275) digested with either *Bam*HI/*Sfu*I or a *Bam*HI/*Hind*III, depending on the restriction sites present in the PCR fragments to be cloned. Ligation was carried out with the Roche Rapid Ligation kit and protocol. The ligation mix was then used to transform *E. coli* HB101 competent cells and transformants were selected in Luria-Broth plates containing ampicillin at 100 μg/ml after an overnight incubation at 37°C. Several colonies of each transformation were picked and inoculated in 3mL of Luria-Broth with ampicillin at 100 μg/ml and incubated at 37°C with shaking overnight.

Plasmid DNA was prepared using 1.5 mL of cultures and the QIAprep Miniprep kit (QIAGEN). Recombinant clones were identified by analytical restriction enzyme analysis with *Bam*HI-*Sal*I. Large-scale preparations of recombinant plasmids were made to perform sequencing to confirm the nucleotide sequence of the cloned parvovirus B19 fragments.

Yeast expression plasmids exhibiting the expected sequence for NS1, VP1 and VP2 were used for yeast transformation as follows. Competent *Saccharomyces cerevisiae* AD3 cells [*Mat a, trp1+, ura3-52, prb1-1122, pep4-3, prc1-407, [cir^0],::pDM15(pGAP/ADR1::G418^R)], leu2(ΔAD)*] were transformed with plasmid DNAs encoding for NS1, VP1 or VP2, cloned as described above. Selection of yeast recombinants was achieved by two rounds of uracil-deficient plates followed by one round of leucine-deficient plates after incubation at 30 °C for 48-72 hours. Cultures were then grown in leucine-deficient media and then in YEP supplemented with 2%

glucose (Pichuantes et al., *Proteins: Struct. Funct. Genet.* (1989) 6:324-337) for 48h before checking expression of the recombinant proteins.

The sequences for the various proteins from two different isolates are shown in Figures 5-10. In particular, Figures 5A (SEQ ID NO:24) and 5B (SEQ ID NO:25) show the NS1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B1. Figures 6A (SEQ ID NO:26) and 6B (SEQ ID NO:27) show the VP1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B1. Figures 7A (SEQ ID NO:28) and 7B (SEQ ID NO:29) show the VP2 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B1. Figures 8A (SEQ ID NO:30) and 8B (SEQ ID NO:31) show the NS1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B6. Figures 9A (SEQ ID NO:32) and 9B (SEQ ID NO:33) show the VP1 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B6. Figures 10A (SEQ ID NO:34) and 10B (SEQ ID NO:35) show the VP2 nucleotide and protein sequences, respectively, from parvovirus B19 clone 2-B6.

Example 5

Detection and Quantitation of Parvovirus B19 DNA by TaqMan™

A sensitive diagnostic method for the detection of parvovirus B19 infection was designed as follows. In particular, TaqMan™ PCR technology was used to detect and quantitate parvovirus B19 DNA. Quantitative PCR requires efficient extraction of nucleic acid. The volume of plasma/serum used for DNA extraction also influences the sensitivity of detection. Two approaches were used to isolate nucleic acid from 0.5 ml of plasma/serum. In particular, DNA was extracted by (a) binding to silica; and (b) annealing to target-specific oligonucleotides.

(a) Isolation of nucleic acid by binding to silica.

In the presence of high concentrations of chaotropic salt such as guanidinium isothiocyanate, nucleic acids bind to silica. Small sized nucleic acids bind more efficiently to silica under conditions of acidic pH. The bound nucleic acids are

efficiently eluted in low salt, alkaline pH buffer at high temperatures. The substitution of magnetized silica for regular silica greatly facilitates washing and elution steps of nucleic acid isolation. A magnetic base was used to capture the nucleic acid-bound silica particles, thus eliminating centrifugations required to sediment regular silica particles.

The lysis buffer used was from Organon-Teknika (Durham, NC). This lysis buffer contains guanidinium isothiocyanate to solubilize proteins and inactivate RNases and DNases. The detergent Triton X-100 further facilitates the process of solubilization and disintegration of cell structure and nuclear proteins, thus releasing nucleic acid. The lysis reagent was acidified to enhance nucleic acid binding, and 50 µl of alkaline elution buffer was used to elute the bound nucleic acid. Following nucleic acid isolation, the presence of parvovirus DNA was determined by performing TaqMan™ PCR, as described below.

(b) Isolation of nucleic acid by annealing to target-specific oligonucleotides.

Although use of magnetized silica greatly facilitates rapid and easy handling during the washing and elution steps, isolation of nucleic acid is still laborious and time consuming. Therefore one-step capture of specific nucleic acid target from plasma or serum using magnetic beads was used. In order to make this applicable for a wide variety of viral nucleic acid capture tests, generic magnetic beads coupled with oligo dT were used. Sera-Mag magnetic oligo (dT) beads (Seradyn, Indianapolis, IN) with an oligo dT length of 14mers were used in combination with Capture oligonucleotides containing 20 poly A's at 3' end contiguous with the parvovirus-specific sequence used (designated at the end of the sequence specified below).

The antisense capture oligonucleotides used were derived from the 700 bp fragment and were as follows:

VSPC1 - AAAAAAAAAAAAAAAAAAAATCCTTAACAGCAATTTCTGATA (nt 3492-3514) (*) (SEQ ID NO:49)

VSPC2 - AAAAAAAAAAAAAAAAAAAACGCCCTGTAGTGCTGTCAG (nt 3549-3568) (SEQ ID NO:50)

VSPC3 - AAAAAAAAAAAAAAAAAAAATATACCCAAATAGGAAGTTCTG (nt 3639-3660) (SEQ ID NO:51)

VSPC4 - AAAAAAAAAAAAAAAAAAAATAAAATGCTGATTCTTCACTTGC (nt 3737-3759) (SEQ ID NO:52)

VSPC5 - AAAAAAAAAAAAAAAAAAAATGCTGTACCTCCTGTACCTA (nt 3789-3808) (SEQ ID NO:53)

VSPC6 - AAAAAAAAAAAAAAAAAAAAAGCCCTCTAAATTTTCTGGG (nt 3838-3857) (SEQ ID NO:54)

VSPC7 - AAAAAAAAAAAAAAAAAAAACTCCTAATGTGTCAGGAACC (nt 3910-3929) (SEQ ID NO:55)

(*) Nucleotide numbers are according to Shade et al., *J. Virol.* (1986) 58:921-936.

The magnetic beads were suspended in Novagen lysis buffer (Madison, WI) and a series of seven capture oligonucleotides (VSPC1-VSPC7, described above) were tested individually or in combination, to capture parvovirus B19 DNA from a panel obtained from the FDA Center for Biologic Evaluation and Research, U.S. Department of Health and Human Services (FDA-CBER).

(c) Washing the beads with a wash buffer.

Following capture, the beads were washed with a buffer containing 10 mM Hepes buffered to pH 7.5 in 0.3 M NaCl., and 0.5% NP-40. After treatment of serum with lysis buffer, hybridization, magnetic adsorption of beads, and removal of lysis buffer, 1.5 ml of the wash buffer was added to the beads. Following the usual vortexing, magnetic adsorption, and removal of the wash buffer, the beads were washed a second time in 0.5 ml of the same buffer, so that the magnetic beads can be

compacted, for easy suspension in 100 ml of Universal PCR buffer containing all the reagents for the Taqman assay. The beads with the captured DNA were transferred to a TaqMan™ plate for detection by TaqMan™ PCR as described below. Several oligonucleotide combinations were efficient at capturing B19 as detected by TaqMan™ assay.

In particular, the TaqMan™ technology amplifies captured target nucleic acid as DNA amplicons. An alternative is amplifying the captured target as RNA. For this, amplification oligonucleotides consisted of a parvovirus B19-specific primer with a T7 promoter sequence, in order to generate RNA amplicons using T7 RNA polymerase. Three amplification primers (VSA1-A3, described below), derived from the 700 bp sequence corresponding to nucleotides 2936-3635 of the parvovirus B19 genome described in Shade et al., *J. Virol.* (1986) 58:921-936 were tested for their ability to amplify. The primers were as follows:

Sense strand amplification primers
VSA1-AATTCTAATACGACTCACTATAGGGAGAAGGCCATATACTCATTGGACTGT (nt 2942-2961) (SEQ ID NO:56)

VSA2 - AATTCTAATACGACTCACTATAGGGAGAAGGCCAGAGCACCATTATAA (nt 3272-3288) (SEQ ID NO:57)

VSA3 -AATTCTAATACGACTCACTATAGGGAGAAGGCACAATGCCAGTGGAAA (nt 3317-3333) (SEQ ID NO:58)

VSP2-GTGCTGAAACTCTAAAGGT (Anti-sense Primer- nt 3424-3442) (SEQ ID NO:59)

RNamplifire kit (Qiagen) reagents were used to examine amplification efficiency using 50 copies of the parvovirus DNA as target in a final volume of 20 mLs. The amplification primers were tested individually or in combination using VSP2 as the second primer. Following one hour incubation at 42 °C as recommended by the manufacturer, an aliquot of the amplified material was diluted 100 fold, for detection by the TaqMan™ assay to assess the efficiency of the amplification primers.

A combination of two amplification primers, VSA2 and VSA3 with VSP2, was highly efficient at generating RNA amplicons.

The sensitivity of the TaqMan™ assay, the suitability of the PCR primers and the optimum reaction conditions were established using plasmid DNA containing the 4.7 kb fragment described above. This fragment includes the VP1 region, as well as the NS1 and VP2 regions (see, Figure 1). PCR amplification primers derived from the VP1 region, as detailed below, were used. The numbering is relative to Shade et al., *J. Virol.* (1986) 58:921-936. X represents 5'-fluorescein phosphoramidite and Z represents DABCYL-dT, both obtained from Glen Research Corporation, Sterling, VA. The numbers designated to the right of the sequence refer to the nucleotides in the primers from the parvovirus B19 sequence.

VSP1- GGAGGCAAAGGTTTGCA (Sense Primer- nt 3334-3350) (SEQ ID NO:60)

VSP2-GTGCTGAAACTCTAAAGGT (Anti-sense Primer-nt 3424-3442) (SEQ ID NO:59)

VSPPR1-XCCCATGGAGATATTTAGATTZ (Probe-nt 3379-3398) (SEQ ID NO:61)

Vpara 8: TCCATATGACCCAGAGCACCA (nt3262-3460) (SEQ ID NO: 88)

Vpara 9: TTTCCACTGGCATTGTGGC (Anti-sense Primer- nt 3313-3331)(SEQ ID NO: 89)

Vpara10: X AGCTAGACCTGCATGTCACTG Z, where X is Fam and Z is Tamra. (nt3286-3310) (SEQ ID NO: 90)

The plasmid DNA concentration was estimated spectrophotometrically, and serial dilution was performed to obtain 5,000-10 copies/20 μl. The reaction mix in a final volume of 50 μl contained 20 μl sample, 1X Gold Taq amplification buffer (Perkin Elmer) with 3.2 mM $MgCl_2$, 300 μM each of dNTPs, 1 pmol each of the amplification primers, 0.4 pmol of the probe, and 1 unit of AmpliTaq enzyme. The reaction conditions included 10 min at 95 °C to activate the enzyme followed by 45 cycles of 30 secs at 95

^{0}C, alternating with 60 ^{0}C in an ABI 7700 Sequence Detector.

Using the primer pair VSP1 and VSP2 which generated a 109 bp PCR product and the probe VSPPR1, as few as 10 copies/assay were detectable. Since the sample volume was 20 μL in a final volume of 50 μLs, this suggests that plasma samples containing as few as 50 copies/ml of parvovirus B19 DNA could be extracted and detected by TaqMan™ technology. Since parvovirus is a high titer virus, plasma/serum volumes of 50 μL could be extracted and used for analysis.

Using the FDA-CBER parvovirus B19 DNA positive sample (10^6 copies/ml) TaqMan™ technology detected as few as 50 copies per assay. In an attempt to correlate the nucleic acid and immunotiter, the viral DNA load was quantitated in several antibody-positive samples.

Accordingly, novel human parvovirus B19 sequences and detection assays using these sequences have been disclosed. From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope thereof.

Claims

1. A method of detecting human parvovirus B19 infection in a biological sample, said method comprising:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein said nucleic acid comprises an RNA target sequence;

(b) reacting the isolated parvovirus B19 nucleic acid with a first oligonucleotide which comprises a first primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the RNA target sequence to complex therewith, wherein said first primer further comprises a promoter for a DNA-dependent RNA polymerase 5' and operably linked to the complexing sequence, wherein said reacting is done under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(c) extending the first primer in an extension reaction using the RNA target sequence as a template to give a first DNA primer extension product complementary to the RNA target sequence;

(d) separating the first DNA primer extension product from the RNA target sequence using an enzyme which selectively degrades the RNA target sequence;

(e) treating the DNA primer extension product with a second oligonucleotide which comprises a second primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the DNA primer extension product to complex therewith under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(f) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product, thereby producing a template for the DNA-dependent RNA polymerase;

(g) using the template to produce multiple RNA copies of the target sequence using a DNA-dependent RNA polymerase which recognizes the promoter sequence; and

(h) using the RNA copies of step (g), autocatalytically repeating steps (b) to (g) to amplify the target sequence.

2. The method of claim 1 further comprising the steps of:

(i) adding a labeled oligonucleotide probe to the product of step (h), wherein said oligonucleotide probe is complementary to a portion of said target sequence, under conditions that provide for the hybridization of said probe with said target sequence to form a probe:target complex; and

(j) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

3. The method of claim 2, wherein said label is an acridinium ester.

4. The method of claim 2, wherein said first and second primers, and said probe are derived from the VP1 region of the human parvovirus B19 genome.

5. The method of claim 4, wherein said first and second primers, and said probe are derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z.

6. The method of claim 1, further comprising providing an internal control in step (b).

7. The method of claim 6, wherein the internal control is derived from the sequence of Figure 12 (SEQ ID NO:92).

8. The method of claim 6, wherein the internal control comprises SEQ ID NO:90.

9. A method of detecting human parvovirus B19 infection in a biological sample, said method comprising:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein said nucleic acid comprises an RNA target sequence;

(b) reacting the isolated parvovirus B19 nucleic acid with a first oligonucleotide which comprises a first primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the RNA target sequence to complex therewith, wherein said first primer further comprises a promoter for a DNA-dependent RNA polymerase 5' and operably linked to the complexing sequence, wherein said first primer comprises a sequence derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z and said reacting is done under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(c) extending the first primer in an extension reaction using the RNA target sequence as a template to give a first DNA primer extension product complementary to the RNA target sequence;

(d) separating the first DNA primer extension product from the RNA target sequence using an enzyme which selectively degrades the RNA target sequence;

(e) treating the DNA primer extension product with a second oligonucleotide which comprises a second primer comprising a complexing sequence sufficiently complementary to the 3'-terminal portion of the DNA primer extension product to complex therewith, wherein said second primer is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z and said treating is done under conditions that provide for the formation of an oligonucleotide/target sequence complex and initiation of DNA synthesis;

(f) extending the 3'-terminus of the second primer in a DNA extension reaction to give a second DNA primer extension product, thereby producing a template for the DNA-dependent RNA polymerase;

(g) using the template to produce multiple RNA copies of the target sequence

using a DNA-dependent RNA polymerase which recognizes the promoter sequence; and (h) using the RNA copies of step (g), autocatalytically repeating steps (b) to (g)

to amplify the target sequence;

(i) adding an acridinium ester-labeled oligonucleotide probe to the product of step (h), wherein said oligonucleotide probe is complementary to a portion of said target sequence and said probe is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z, wherein said probe is added under conditions that provide for the hybridization of said probe with said target sequence to form a probe:target complex; and

(j) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

10. The method of claim 9, further comprising providing an internal control in step (b).

11. The method of claim 10, wherein the internal control is derived from the sequence of Figure 12 (SEQ ID NO:92).

12. The method of claim 10, wherein the internal control comprises SEQ ID NO:90.

13. A method for amplifying a target parvovirus B19 nucleotide sequence, said method comprising:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein said nucleic acid comprises an RNA target sequence;

(b) adding one or more primers capable of hybridizing to the RNA target sequence, wherein said one or more primers are derived from the polynucleotide sequences depicted in any one of Figures 2A-2U and Figures 11A-11Z;

(c) adding an oligonucleotide probe capable of hybridizing to the RNA target sequence 3' relative to the one or more primers;

(d) extending the one or more primers using a polymerase.

14. The method of claim 13, wherein the RNA target sequence of step (a) is reverse transcribed to provide cDNA.

15. The method of claim 14, further comprising amplifying the cDNA using polymerase chain reaction (RT-PCR) or asymmetric gap ligase chain reaction (RT-AGLCR).

16. The method of claim 13, wherein the polymerase is a thermostable polymerase.

17. The method of claim 16, wherein the thermostable polymerase is Taq polymerase or Vent polymerase.

18. The method of claim 13, wherein the polymerase is *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, or T4 DNA polymerase.

19. The method of claim 13, further comprising providing an internal control in step (b).

20. The method of claim 19, wherein the internal control is derived from the sequence of Figure 12 (SEQ ID NO:92).

21. The method of claim 19, wherein the internal control comprises SEQ ID NO:90.

22. A polynucleotide comprising a nucleotide sequence comprising any one of the nucleotide sequences depicted in Figures 2A-2U or Figures 11A-11Z.

23. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2A.

24. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2B.

25. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2C.

26. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2D.

27. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2E.

28. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2F.

29. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2G.

30. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2H.

31. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2I.

32. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2J.

33. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2K.

34. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2L.

35. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2M.

36. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2N.

37. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2O.

38. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2P.

39. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2Q.

40. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2R.

41. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2S.

42. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2T.

43. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 2U.

44. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11A.

45. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11B.

46. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11C.

47. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11D.

48. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11E.

49. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11F.

50. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11G.

51. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11H.

52. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11I.

53. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11J.

54. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11K.

55. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11L.

56. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11M.

57. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11N.

58. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11O.

59. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11P.

60. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11Q.

61. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11R.

62. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11S.

63. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11T.

64. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11U.

65. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11V.

66. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11W.

67. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11X.

68. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11Y.

69. The polynucleotide of claim 22, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figure 11Z.

70. A polynucleotide comprising a nucleotide sequence comprising any one of the nucleotide sequences depicted in Figures 3A-3C or 4A-4C.

71. The polynucleotide of claim 70, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figures 3A-3C.

72. The polynucleotide of claim 70, wherein said nucleotide sequence consists of the nucleotide sequence depicted in Figures 4A-4C.

73. An oligonucleotide primer consisting of a promoter region recognized by a DNA-dependent RNA polymerase operably linked to a human parvovirus B19-specific complexing sequence of about 10 to about 75 nucleotides.

74. The oligonucleotide primer of claim 73, wherein said promoter region is the T7 promoter and said polymerase is T7 RNA polymerase.

75. The oligonucleotide primer of claim 73, wherein said human parvovirus B19-specific sequence is from the VP1 region of the human parvovirus B19 genome.

76. The oligonucleotide primer of claim 75, wherein said human parvovirus B19-specific sequence is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U.

77. An oligonucleotide primer consisting of a T7 promoter operably linked to a human parvovirus B19-specific complexing sequence of about 10 to about 75 nucleotides, wherein said human parvovirus B19-specific complexing sequence is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z.

78. An oligonucleotide probe comprising a parvovirus B19-specific hybridizing sequence of about 10 to about 50 nucleotides linked to an acridinium ester label.

79. The oligonucleotide probe of claim 78, wherein said human parvovirus B19-specific hybridizing sequence is from the VP1 region of the human parvovirus B19 genome.

80. The oligonucleotide probe of claim 79, wherein said human parvovirus B19-specific hybridizing sequence is derived from the polynucleotide sequence depicted in any one of Figures 2A-2U or Figures 11A-11Z.

81. A diagnostic test kit comprising an oligonucleotide primer according to claim 73, and instructions for conducting the diagnostic test.

82. The diagnostic test kit of claim 81, further comprising an oligonucleotide probe comprising a parvovirus B19-specific hybridizing sequence of about 10 to about 50 nucleotides linked to an acridinium ester label.

83. A method for detecting human parvovirus B19 infection in a biological sample, said method comprising:

(a) isolating nucleic acid from a biological sample suspected of containing human parvovirus B19 DNA, wherein said nucleic acid comprises a target sequence;

(b) reacting the isolated parvovirus B19 nucleic acid with a detectably labeled probe sufficiently complementary to and capable of hybridizing with the target sequence, wherein the probe is derived from the polynucleotide sequences depicted in any one of Figures 2A-2U and Figures 11A-11Z, and further wherein said reacting is done under conditions that provide for the formation of a probe/target sequence complex; and

(c) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

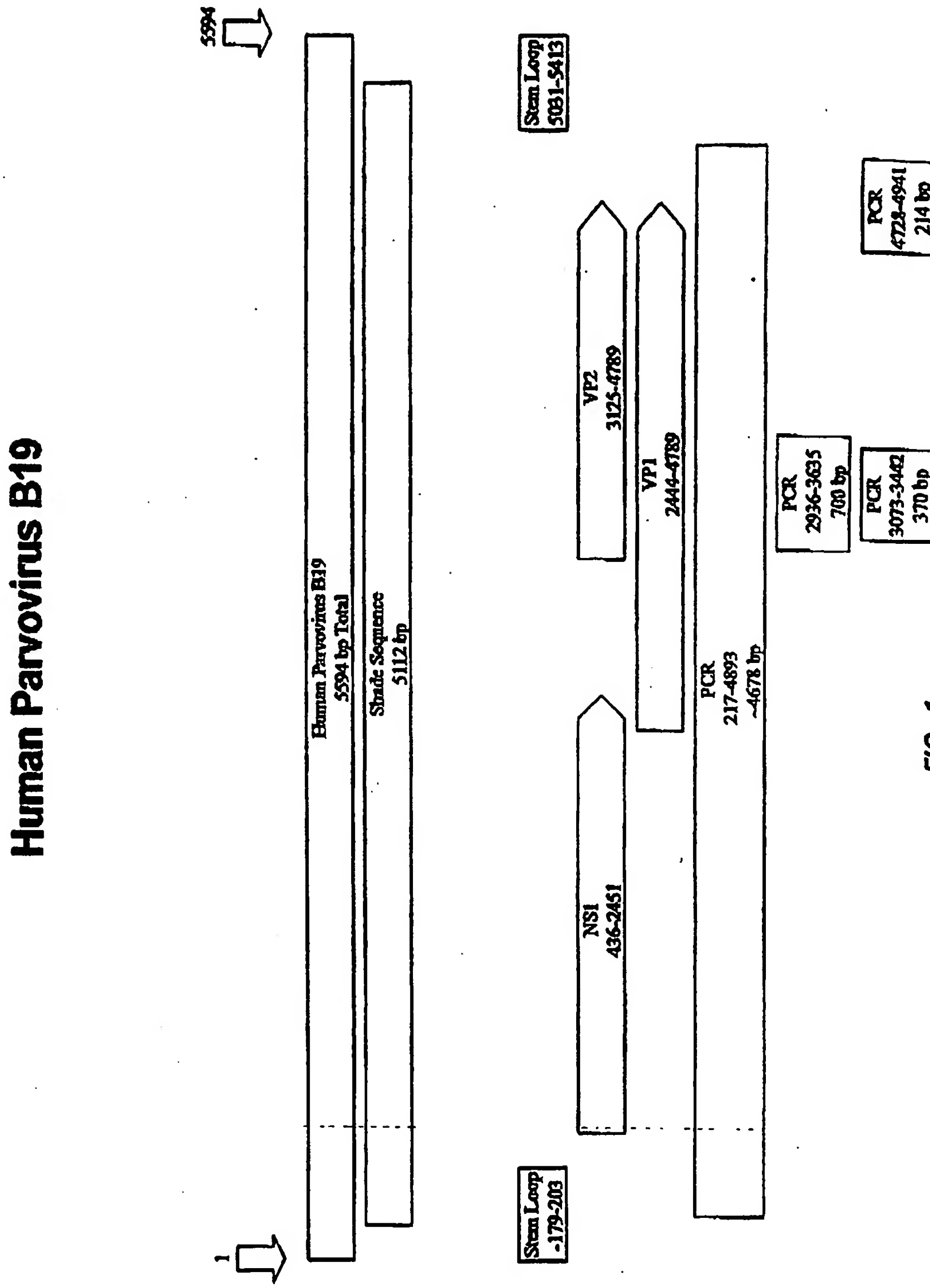
Human Parvovirus B19
5594
1
Human Parvovirus B19
5594 bp Total
Shade Sequence
5112 bp
Stem Loop
5031-5413
Stem Loop
-179-203
VP2
3125-4789
VP1
2444-4789
NS1
436-2451
PCR
217-4893
~4678 bp
PCR
4728-4941
214 bp
PCR
2936-3635
700 bp
PCR
3073-3442
370 bp

FIG. 1

CH47-26

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgttaaaagcatgtggagtgaggggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttgtttttttcacctttagagtttcagcatttaattgaaaact
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gagggggagtacaagttactgacagcactaccgggcgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2A

CH48-29

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaacaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggtggcagtaatcctgccaaaagcatgtggagtgaggggggccacttttactgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gctagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactca
actccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcacctaattgaaaattat
ggaagtatagctcctgatgatttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactgg
aggggggggtacaggttactgacagcactacagggcgcctatgcctgttagtagaccatgaatacaagtacc
catatgtgttagggcaaggtcaggatactttag

FIG. 2B

CH33-2

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaacaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggtggcagtaatcctgccaaaagcatgtggagtgagggggccacttttactgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gctagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactca
actccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcacctaattgaaaattat
ggaagtatagctcctgatgatttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactgg
agggggggtacaggttactgacagcactacagggcgcctatgcttgttagtagaccatgaatacaagtacc
catatgtgttagggcaaggtcaggatactttag

FIG. 2C

CH33-3

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaacatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggtggcagtaatcctgccaaaagcatgtggagtgagggggccacttttactgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gctagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactca
actccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcacctaattgaaaattat
~~ggaagtatagctcctgatgatttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactgg~~
agggggggtacaggttactgacagcactacagggcgcctatgcctgttagtagaccatgaatacaagtacc
catatgtgttagggcaaggtcaggatactttag

FIG. 2D

CH33-4

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggtggcagtaatcctgccaaaagcatgtggagtgagggggccacttttactgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gctagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactca
actccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacctaattgaaaattat
ggaagtatagctcctgatgatttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactgg
aggggggtacaggttactgacagcactacagggcgcctatgcctgttagtagaccatgaatacaagtacc
catatgtgttagggcaaggtcaggatactttag

FIG. 2E

CH42-7

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtcaaaagcatgtggagtgagggggccacttttagtgccaac
tctgtaacttgtacattttccaggcagtttttaattccatatgacccagagcaccattataaggtgttttctcccgc
agcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatact
caaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaat
tatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaact
ggaggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagta
cccatatgtgttagggcaaggtcaggatactttag

FIG. 2F

CH42-18

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgtcaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgc
agcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatact
caaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaat
tatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaact
ggaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagta
cccatatgtgttagggcaaggtcaggatactttag

FIG. 2G

CH42-19

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgtcaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgc
agcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatact
caaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaat
tatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaact
ggaggggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagta
cccatatgtgttagggcaaggtcaggatactttag

FIG. 2H

CH46-23

attaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
ncacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtcaaaagcatgtggagtgagggggccacttttagtgccaac
tctgtaacttgtacattttccaggcagtttttaattccatatgacccagagcaccattataaggtgttttctcccgc
agcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatact
caaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaat
tatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaact
ggagggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagta
cccatatgtgttagggcaaggtcaggatactttag

FIG. 2I

CH1-1

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtcaaaagcatgtggagtgagggggccacttttagtgccaac
tctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgc
agcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatact
caaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaat
tatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaact
ggagggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagta
cccatatgtgttagggcaaggtcaggatactttag

FIG. 2J

CH1-6

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtcaaaagcatgtggagtgaggggccacttttagtgccaac
tctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgc
agcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatact
caaccccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcacttaattgaaaat
tatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaact
ggagggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagta
cccatatgtgttagggcaaggtcaggatactttag

FIG. 2K

CH2-8

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtgaaaagcatgtggagtgaggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gagggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2L

CH2-10

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgtgaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacagaactg
gaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2M

H2-11C

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgtgaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2N

CH5-13

ctaaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgttaaaagcatgtggagtgagggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaagaggcaaaggtttgcactattagtcccataatgggatactca
accccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaattat
ggcagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactgg
agggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagtacc
caatgtgttagggcaaggtcaggatactttag

FIG. 2O

CH7-22

ataaatccatgtactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgttaaaagcatgtggagtgagggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttgttttttcacctttagagtttcagcatttaattgaaaact
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gagggggagtacaagttactgacagcactaccgggcgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2P

CH13-27

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaattctgtcaaaagcatgtggagtgaggggggccacttttagtgctaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcgagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccatcagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gaggggggggtacaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2Q

CH14-33

ataaatccatatactcattggactgtggcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggagtaatcctgttaaaagcatgtggagtgaggggggccacttttagtgccaactc
tgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgcag
caagtagctgccacaatgccagtggaaaagaggcaaaggtttgcaccattagtcccataatgggatactcaa
ccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaattatg
gtagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaagatgttacagacaaaactggag
gggggtacaggttactgacagcactacagggcgcctatgcatgttagtggaccatgaatacaagtaccca
tatgtgttagggcaaggtcaggatactttag

FIG. 2R

CH62-2

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcaattaattctgcagaagccagcact
ggtgcaggaggggggggcagtaatcctgtcaaaagcatgtggagtgaggggggccacttttagtgccaact
ctgtaacttgtacakttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gccagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gagggggggtacaggttactgacagcactacaggccgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2S

CH64-2

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgttaaaagcatgtggagtgaggggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttcgcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatacttagattttaatgctttaaatttatttttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacggacaaaactg
gagggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2T

CH67-2

ataaatccatatactcattggactgtggcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggggagtaatcctgttaaaagcatgtggagtgagggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaagaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaagatgttacagacaaaactg
gagggggggtacaggttactgacagcactacagggcgcctatgcatgttagtggaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 2U

Parvovirus B19 clone #2-B1

1 cccgccttat gcaaatgggc agccatctta agtgttttac tataatttta ttggtcagtt
61 ttgtaacggt taaaatgggc ggagcgtagg caaggactac agtatatata gcacagcact
121 gccgcagctc tttctttctg ggctgctttt ttcctggact tacttgctgt tttttgtgag
181 ctaactaaca ggtatttata ctacttgtta acatactaac atggagctat ttagaggggt
241 gcttcaagtt tcttctaatg ttctggactg tgctaacgat aactggtggt gctctttact
301 ggatttagac acttctgact gggaaccact aactcatact aacagactaa tggcaatata
361 cttaagcagt gtggcttcta agcttgactt tactgggggg ccactagcag ggtgcttgta
421 ctttttcaa gtagaatgta acaaatttga agaaggctat catattcatg tggttattgg
481 ggggccaggg ttaaacccca gaaacctcac agtgtgtgta gaggggttat ttaataatgt
541 actttatcac cttgtaactg aaaatctgaa gctaaaattt ttgccaggaa tgactacaaa
601 aggcaaatac tttagagatg gagagcagtt tatagaaaac tatttaatga aaaaaatacc
661 tttaaatgtt gtatggtgtg ttactaatat tgatggacat atagatacct gtatttctgc
721 tacttttaga aagggagctt gccatgccaa gaaaccccgc atcaccacag ccataaatga
781 tactagtact gatgctgggg agtctagcgg cacaggggca gaggttgtgc catttaatgg
841 gaagggaact aaggctagca taaagtttca aactatggta aactggttgt gtgaaaacag
901 agtgtttaca gaggataagt ggaaactagt tgactttaac cagtacactt tactaagcag
961 tagtcacagt ggaagttttc aaattcaaag tgcactaaaa ctagcaattt ataaagcaac
1021 taatttagtg cctactagca catttttatt gcatacagac tttgagcaag ttatgtgtat
1081 taaaaacaat aaaattgtta aattgttact ttgtcaaaac tatgaccccc tattagtggg
1141 gcagcatgtg ttaaagtgga ttgataaaaa atgtggcaag aaaaacacac tgtggtttta
1201 tgggccgcca agtacaggga aaacaaactt ggcaatggcc attgctaaaa gtgttccagt
1261 atatggcatg gttaactgga ataatgaaaa ctttccattt aatgatgtag caggaaaaag
1321 cttggtggtc tgggatgaag gtattattaa gtctacaatt gtagaagctg caaaagccat
1381 tttaggcggg caacccacca gggtagatca aaaaatgcgt ggaagtgtag ctgtgcctgg
1441 agtacctgtg gttataacca gcaatggtga cattactttt gttgtaagcg ggaacactac
1501 aacaactgta catgctaaag ccttaaaaga gcgcatggta aagttaaact ttactgtaag
1561 atgcagccct gacatggggt tactaacaga ggctgatgta caacagtggc ttacatggtg
1621 taatgcacaa agctgggacc actatgaaaa ctgggcaata aactacactt ttgatttccc
1681 tggaattaat gcagatgccc tccacccaga cctccaaacc accccaattg tcacagacac
1741 cagtatcagc agcagtggtg gtgaaagctc tgaagaactc agtgaaagca gctttttaa
1801 cctcatcacc ccaggcgcct ggaacactga aaccccgcgc tctagtacgc ccatccccgg
1861 gaccagttca ggagaatcat ctgtcggaag cccagtttcc tccgaagttg tagctgcatc
1921 gtgggaagaa gccttctaca cacctttggc agaccagttt cgtgaactgt tagttggggt
1981 tgattatgtg tgggacggtg taaggggttt acctgtctgt tgtgtgcaac atattaacaa

FIG. 3A

2041 tagtggggga ggcttgggac tttgtcccca ttgcattaat gtaggggctt ggtataatgg
2101 atggaaattt cgagaattta ccccagattt ggtgcgatgt agctgccatg tgggagcttc
2161 taatcccttt tctgtgctaa cctgcaaaaa atgtgcttac ctgtctggat tgcaaagctt
2221 tgtagattat gagtaaagaa agtggcaaat ggtgggaaag tgatgataaa tttgctaaag
2281 ctgtgtatca gcaatttgtg gaattttatg aaaaggttac tggaacagac ttagagctta
2341 ttcaaatatt aaaagatcat tataatattt ctttagataa tcccctagaa aacccatcct
2401 ctttgtttga cttagttgct cgtattaaaa ataaccttaa aaactctcca gacttatata
2461 gtcatcattt tcaaagtcat ggacagttat ctgaccaccc ccatgcctta tcatccagta
2521 gcagtcatgc agaacctaga ggagaagatg cagtattatc tagtgaagac ttacacaagc
2581 ctgggcaagt tagcgtacaa ctacccggta ctaactatgt tgggcctggc aatgagctac
2641 aagctgggcc cccgcaaagt gctgttgaca gtgctgcaag gattcatgac tttaggtata
2701 gccaactggc taagttggga ataaatccat atactcattg gactgtagca gatgaagagc
2761 ttttaaaaaa tataaaaaat gaaactgggt ttcaagcaca agtagtaaaa gactacttta
2821 ctttaaaagg tgcagctgcc cctgtggccc attttcaagg aagtttgccg gaagttcccg
2881 cttacaacgc ctcagaaaaa tacccaagca tgacttcagt taattctgca gaagccagca
2941 ctggtgcagg aggggggggc agtaatcctg tgaaaagcat gtggagtgag gggggccactt
3001 ttagtgccaa ctctgtaact tgtacatttt ccagacaatt tttaattcca tatgacccag
3061 agcaccatta taaggtgttt tctcccgcag caagtagctg ccacaatgcc agtggaaagg
3121 aggcaaaggt ttgcaccatt agtcccataa tgggatactc aaccccatgg agatatttag
3181 attttaatgc tttaaattta ttttttcac ctttagagtt tcagcactta attgaaaatt
3241 atggaagtat agctcctgat gctttaactg taaccatatc agaaattgct gttaaggatg
3301 ttacggacaa aactggaggg ggggtgcagg ttactgacag cactacaggg cgcctatgca
3361 tgttagtaga ccatgaatat aagtacccat atgtgttagg gcaaggtcaa gatactttag
3421 ccccagaact tcctatttgg gtatactttc cccctcaata cgcttactta acagtaggag
3481 atgttaacac acaaggaatt tctggagaca gcaaaaaatt ggcaagtgaa gaatcagcat
3541 tttatgtttt ggaacacagt tcttttcagc ttttaggtac aggaggtaca gcaactatgt
3601 cttataagtt tcctccagtg cccccagaaa atttagaggg ctgcagtcaa cacttttatg
3661 aaatgtacaa ccccttatac ggatcccgct taggggttcc tgacacatta ggaggtgacc
3721 caaaatttag atctttaaca catgaagacc atgcaattca gccccaaaac ttcatgccag
3781 ggccactagt aaactcagtg tctacaaagg agggagacag ctctagtact ggagctggaa
3841 aagccttaac aggccttagc acaggtacct ctcaaaacac tagaatatcc ttacgccctg
3901 ggccagtgtc tcagccgtac caccactggg acacagataa atatgtcaca ggaataaatg
3961 ccatttctca tggtcagacc acttatggta acgctgaaga caaagagtat cagcaaggag
~~4021 tgggtagatt tccaaatgaa aaagaacagc taaaacagtt acagggttta aacatgcaca~~
4081 cctactttcc caataaagga acccagcaat atacagatca aattgagcgc cccctaatgg
4141 tgggttctgt atggaacaga agagcccttc actatgaaag ccagctgtgg agtaaaattc
4201 caaatttaga tgacagtttt aaaactcagt ttgcagcctt aggaggatgg ggtttgcatc

FIG. 3B

4261 agccacctcc tcaaatattt ttaaaaatat taccacaaag tgggccaatt ggaggtatta
4321 aatcaatggg aattactacc ttagttcagt atgccgtggg aattatgaca gtaaccatga
4381 catttaaatt ggggccccgt aaagctacgg gacggtggaa tcctcaacct ggagtgtatc
4441 ccccgcacgc agcaggtcat ttaccatatg tactatatga ccccacagct acagatgcaa
4501 aacaacacca cagacatgga tatgaaaagc ctgaagaatt gtggacagcc aaaagccgtg
4561 tgcacccatt gtaaacactc cccaccgtgc cctcagccag gatgtgtaac taaacgccca
4621 ccagtaccac ccagactgta cctgcccccct cctataccta taagacagcc taacacaa

FIG. 3C

Parvovirus B19 clone #2-B6

1 cccgccttat gcaaatgggc agccatctta agtgttttac tataatttta ttggtcagtt
61 ttgtaacggt taaaatgggc ggagcgtagg caaggactac agtatatata gcacagcact
121 gccgcagctc tttctttctg ggctgctttt ttcctggact tacttgctgt ttttgtgag
181 ctaactaaca ggtatttata ctacttgtta acatactaac atggagctat ttagaggggt
241 gcttcaagtt tcttctaatg ttctggactg tgctaacgat aactggtggt gctctttact
301 ggatttagac acttctgact gggaaccact aactcatact aacagactaa tggcaatata
361 cttaagcagt gtggcttcta agcttgactt tactgggggg ccactagcag ggtgcttgta
421 ctttttcaa gtagaatgta acaaatttga agaaggctat catattcatg tggttattgg
481 ggggccaggg ttaaacccca gaaacctcac agtgtgtgta gaggggttat ttaataatgt
541 actttatcac cttgtaactg aaaatctgaa gctaaaattt ttgccaggaa tgactacaaa
601 aggcaaatac tttagagatg gagagcagtt tatagaaaac tatttaatga aaaaaatacc
661 tttaaatgtt gtatggtgtg ttactaatat tgatggacat atagatacct gtatttctgc
721 tacttttaga aagggagctt gccatgccaa gaaaccccgc atcaccacag ccataaatga
781 tactagtact gatgctgggg agtctagcgg cacaggggca gaggttgtgc catttaatgg
841 gaagggaact aaggctagca taaagtttca aactatggta aactggttgt gtgaaaacag
901 agtgtttaca gaggataagt ggaaactagt tgactttaac cagtacactt tactaagcag
961 tagtcacagt ggaagttttc aaattcaaag tgcactaaaa ctagcaattt ataaagcaac
1021 taatttagtg cctactagca catttttatt gcatacagac tttgagcaag ttatgtgtat
1081 taaagacaat aaaattgtta aattgttact ttgtcaaaac tatgaccccc tattagtggg
1141 gcagcatgtg ttaaagtgga ttgataaaaa atgtggcaag aaaaacacac tgtggtttta
1201 tggaccgcca agtacaggga aaacaaactt ggcaatggcc attgctaaaa gtgttccagt
1261 atatggcatg gttaactgga ataatgaaaa ctttccattt aatgatgtag caggaaaaag
1321 cttggtggtc tgggatgaag gtattattaa gtctacaatt gtagaagctg caaaagccat
1381 tttaggcggg caacccacca gggtagatca aaaaatgcgt ggaagtgtag ctgtgcctgg
1441 agtacccgtg gttataacca gcaatggtga cattactttt gttgtaagcg ggaacactac
1501 aacaactgta catgctaaag ccttaaaaga gcgcatggta aagttaaact ttactgtaag
1561 atgcagccct gacatggggt tactaacaga ggctgatgta caacagtggc ttacatggtg
1621 taatgcacaa agctgggacc actatgaaaa ctgggcaata aactacactt ttgatttccc
1681 tggaattaat gcagatgccc tccacccaga cctccaaacc accccaattg tcacagacac
1741 cagtatcagc agcagtggtg gtgaaagctc tgaagaactc agtgaaagca gcttttttaa
1801 cctcatcacc ccaggcgcct ggaacactga aaccccgcgc tctagtacgc ccatccccgg
~~1861 gaccagttca ggagaatcat ctgtcggaag cccagtttcc tccgaagttg tagctgcatc~~
1921 gtgggaagaa gccttctaca cacctttggc agaccagttt cgtgaactgt tagttggggt
1981 tgattatgtg tgggacggtg taaggggttt acctgtctgt tgtgtgcaac atattaacaa
2041 tagtggggga ggcttgggac tttgtcccca ttgcattaat gtaggggctt ggtataatgg

FIG. 4A

2101 atggaaattt cgagaattta ccccagattt ggtgcgatgt agctgccatg tgggagcttc
2161 taatcccttt tctgtgctaa cctgcaaaaa atgtgcttac ctgtctggat tgcaaagctt
2221 tgtagattat gagtaaagaa agtggcaaat ggtgggaaag tgatgataaa tttgctaaag
2281 ctgtgtatca gcaatttgtg gaattttatg aaaaggttac tggaacagac ttagagctta
2341 ttcaaatatt aaaagatcat tataatattt ctttagataa tcccctagaa aacccatcct
2401 ctttgtttga cttagttgct cgtattaaaa ataaccttaa aaactctcca gacttatata
2461 gtcatcattt tcaaagtcat ggacagttat ctgaccaccc ccatgcctta tcatccagta
2521 gcagtcatgc agaacctaga ggagaagatg cagtattatc tagtgaagac ttacacaagc
2581 ctgggcaagt tagcgtacaa ctacccggta ctaactatgt tgggcctggc aatgagctac
2641 aagctgggcc cccgcaaagt gctgttgaca gtgctgcaag gattcatgac tttaggtata
2701 gccaactggc taagttggga ataaatccat atactcattg gactgtagca gatgaagagc
2761 ttttaaaaaa tataaaaaat gaaactgggt ttcaagcaca agtagtaaaa gactacttta
2821 ctttaaaagg tgcagctgcc cctgtggccc attttcaagg aagtttgccg gaagttcccg
2881 cttacaacgc ctcagaaaaa tacccaagca tgacttcagt taattctgca gaagccagca
2941 ctggtgcagg aggggggggc agtaatcctg tgaaaagcat gtggagtgag gggggccactt
3001 ttagtgccaa ctctgtaact tgtacatttt ccagacaatt tttaattcca tatgacccag
3061 agcaccatta taaggtgttt tctcccgcag caagtagctg ccacaatgcc agtggaaagg
3121 aggcaaaggt ttgcaccatt agtcccataa tgggatactc aaccccatgg agatatttag
3181 attttaatgc tttaaattta tttttttcac ctttagagtt tcagcactta attgaaaatt
3241 atggaagtat agctcctgat gctttaactg taaccatatc agaaattgct gttaaggatg
3301 ttacaaacaa aactggaggg ggggtgcagg ttactgacag cactacaggg cgcctatgca
3361 tgttagtaga ccatgaatat aagtacccat atgtgttagg gcaaggtcaa gatactttag
3421 ccccagaact tcctatttgg gtatactttc cccctcaata cgcttactta acagtaggag
3481 atgttaacac acaaggaatt tctggagaca gcaaaaaatt ggcaagtgaa gaatcagcat
3541 tttatgtttt ggaacacagt tcttttcagc ttttaggtac aggaggtaca gcaactatgt
3601 cttataagtt tcctccagtg cccccagaaa atttagaggg ctgcagtcaa cacttttatg
3661 aaatgtacaa ccccttatac ggatcccgct taggggttcc tgacacatta ggaggtgacc
3721 caaaatttag atctttaaca catgaagacc atgcaattca gccccaaaac ttcatgccag
3781 ggccactagt aaactcagtg tctacaaagg agggagacag ctctagtact ggagctggaa
3841 aagccttaac aggccttagc acaggtacct ctcaaaacac tagaatatcc ttacgccctg
3901 ggccagtgtc tcagccgtac caccactggg acacagataa atatgtcaca ggaataaatg
3961 ccatttctca tggtcagacc acttatggta acgctgaaga caaagagtat cagcaaggag
4021 tgggtagatt tccaaatgaa aaagaacagc taaaacagtt acagggttta aacatgcaca
~~4081 cctactttcc caataaagga acccagcaat atacagatca aattgagcgc cccctaatgg~~
4141 tgggttctgt atggaacaga agagcccttc actatgaaag ccagctgtgg agtaaaattc
4201 caaatttaga tgacagtttt aaaactcagt ttgcagcctt aggaggatgg ggtttgcatc
4261 agccacctcc tcaaatattt ttaaaaatat taccacaaag tgggccaatt ggaggtatta

FIG. 4B

4321 aatcaatggg aattactacc ttagttcagt atgccgtggg aattatgaca gtaaccatga
4381 catttaaatt ggggccccgt aaagctacgg gacggtggaa tcctcaacct ggagtgtatc
4441 ccccgcacgc agcaggtcat ttaccatatg tactatatga ccccacagct acagatgcaa
4501 aacaacacca cagacatgga tatgaaaagc ctgaagaatt gtggacagcc aaaagccgtg
4561 tgcacccatt gtaaacactc cccaccgtgc cctcagccag gatgtgtaac taaacgccca
4621 ccagtaccac ccagactgta cctgcccccct cctataccta taagacagcc taacacaa

FIG. 4C

Clone B1-NS1 single stranded DNA sequence:

atactcttcgaacaaaacaaaatggagctatttagaggggtgcttcaagtttcttctaatgttctggactgtgctaacgataactggtggtgctctt
tactggatttagacacttctgactgggaaccactaactcatactaacagactaatggcaatatacttaagcagtgtggcttctaagcttgacttta
ctgggggccactagcagggtgcttgtactttttcaagtagaatgtaacaaatttgaagaaggctatcatattcatgtggttattgggggcca
gggttaaaccccagaaacctcacagtgtgtgtagaggggttatttaataatgtactttatcaccttgtaactgaaaatctgaagctaaaatttttgc
caggaatgactacaaaaggcaaatactttagagatggagagcagtttatagaaaactatttaatgaaaaaaatacctttaaatgttgtatggtgt
gttactaatattgatggacatatagatacctgtatttctgctacttttagaaagggagcttgccatgccaagaaaccccgcatcaccacagccat
aaatgatactagtactgatgctggggagtctagcggcacagggcagaggttgtgccatttaatgggaagggaactaaggctagcataaag
tttcaaactatggtaaactggttgtgtgaaaacagagtgtttacagaggataagtggaaactagttgactttaaccagtacactttactaagcagt
agtcacagtggaagttttcaaattcaaagtgcactaaaactagcaatttataaagcaactaatttagtgcctactagcacatttttattgcatacag
actttgagcaagttatgtgtattaaaaacaataaaattgttaaattgttactttgtcaaaactatgacccccctattagtggggcagcatgtgttaaag
tggattgataaaaaaatgtggcaagaaaaacacactgtggttttatgggccgccaagtacagggaaaacaaacttggcaatggccattgctaa
aagtgttccagtatatggcatggttaactggaataatgaaaactttccatttaatgatgtagcaggaaaaagcttggtggtctgggatgaag
gtattattaagtctacaattgtagaagctgcaaaagccattttaggcggcaacccaccagggtagatcaaaaaatgcgtggaagtgtagctg
tgcctggagtacctgtggttataaccagcaatggtgacattacttttgttgtaagcgggaacactacaacaactgtacatgctaaagccttaaaa
gagcgcatggtaaagttaaactttactgtaagatgcagccctgacatggggttactaacagaggctgatgtacaacagtggcttacatggtgt
aatgcacaaagctgggaccactatgaaaactgggcaataaactacacttttgatttccctggaattaatgcagatgccctccacccagacctcc
aaaccaccccaattgtcacagacaccagtatcagcagcagtggtggtgaaagctctgaagaactcagtgaaagcagctttttttaacctcatca
ccccaggcgcctggaacactgaaaccccgcgctctagtacgcccatccccgggaccagttcaggagaatcatctgtcggaagcccagtttc
ctccgaagttgtagctgcatcgtgggaagaagccttctacacacctttggcagaccagtttcgtgaactgttagttggggttgattatgtgtggg
acggtgtaaggggtttacctgtctgttgtgtgcaacatattaacaatagtgggggaggcttgggactttgtccccattgcattaatgtaggggct
tggtataatggatggaaatttcgagaatttaccccagatttggtgcgatgtagctgccatgtgggagcttctaatccctttctgtgctaacctgca
aaaaatgtgcttacctgtctggattgcaaagctttgtagattatgagtaagtcgacatactc

FIG. 5A

Clone B1 NS1 amino acid sequence:

MELFRGVLQVSSNVLDCANDNWWCSLLDLDTSDWEPLTHTNRLMAIYLSSVAS
KLDFTGGPLAGCLYFFQVECNKFEEGYHIHVVIGGPGLNPRNLTVCVEGLFNNVLYHLVT
ENLKLKFLPGMTTKGKYFRDGEQFIENYLMKKIPLNVVWCVTNIDGHIDTCISATFRKGA
CHAKKPRITTAINDTSTDAGESSGTGAEVVPFNGKGTKASIKFQTMVNWLCENRVFTEDK
WKLVDFNQYTLLSSSHSGSFQIQSALKLAIYKATNLVPTSTFLLHTDFEQVMCIKNNKIV
KLLLCQNYDPLLVGQHVLKWIDKKCGKKNTLWFYGPPSTGKTNLAMAIAKSVPVYGMVNW
NNENFPFNDVAGKSLVVWDEGIIKSTIVEAAKAILGGQPTRVDQKMRGSVAVPGVPVVIT
SNGDITFVVSGNTTTTVHAKALKERMVKLNFTVRCSPDMGLLTEADVQQWLTWCNAQSWD
HYENWAINYTFDFPGINADALHPDLQTTPIVTDTSISSSGGESSEELSESSFFNLITPGA
WNTETPRSSTPIPGTSSGESSVGSPVSSEVVAASWEEAFYTPLADQFRELLVGVDYVWDG
VRGLPVCCVQHINNSGGGLGLCPHCINVGAWYNGWKFREFTPDLVRCSCHVGASNPFSVL
TCKKCAYLSGLQSFVDYE

FIG. 5B

B1 VP1 single stranded DNA sequence:

atactcaagcttacaaaacaaaatgagtaaagaaagtggcaaatggtgggaaagtgatgataaatttgctaaagctgtgtatcagcaatttgtggaatttta
tgaaaaggttactggaacagacttagagcttattcaaatattaaaagatcattataatatttctttagataatcccctag
aaaacccatcctctttgtttgacttagttgctcgtattaaaaataaccttaaaaactctccagacttatatagtcatcat
tttcaaagtcatggacagttatctgaccacccccatgccttatcatccagtagcagtcatgcagaacctagaggagaaga
tgcagtattatctagtgaagacttacacaagcctgggcaagttagcgtacaactacccggtactaactatgttgggcctg
gcaatgagctacaagctgggcccccgcaaagtgctgttgacagtgctgcaaggattcatgactttaggtatagccaactg
gctaagttgggaataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgg
gtttcaagcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgc
cggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcactggtgca
ggaggggggggcagtaatcctgtgaaaagcatgtggagtgagggggccacttttagtgccaactctgtaacttgtacatt
ttccagacaattttaattccatatgacccagagcaccattataaggtgttttctcccgcagcaagtagctgccacaatg
ccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactcaaccccatggagatatttagattttaat
gctttaaatttatttttttcacctttagagtttcagcacttaattgaaaattatggaagtatagctcctgatgctttaac
tgtaaccatatcagaaattgctgttaaggatgttacggacaaaactggaggggggggtgcaggttactgacagcactacag
ggcgcctatgcatgttagtagaccatgaatataagtacccatatgtgttagggcaaggtcaagatactttagccccagaa
cttcctatttgggtatactttccccctcaatacgcttacttaacagtaggagatgttaacacacaaggaatttctggaga
cagcaaaaaattggcaagtgaagaatcagcattttatgttttggaacacagttcttttcagcttttaggtacaggaggta
cagcaactatgtcttataagtttcctccagtgcccccagaaaatttagagggctgcagtcaacacttttatgaaatgtac
aaccccttatacggatcccgcttaggggttcctgacacattaggaggtgacccaaaatttagatctttaacacatgaaga
ccatgcaattcagccccaaaacttcatgccagggccactagtaaactcagtgtctacaaaggagggagacagctctagta
ctggagctggaaaagccttaacaggccttagcacaggtacctctcaaaacactagaatatccttacgccctgggccagtg
tctcagccgtaccaccactgggacacagataaatatgtcacaggaataaatgccatttctcatggtcagaccacttatgg
taacgctgaagacaaagagtatcagcaaggagtgggtagatttccaaatgaaaaagaacagctaaaacagttacagggtt
taaacatgcacacctactttcccaataaaggaacccagcaatatacagatcaaattgagcgcccccctaatggtgggttct
gtatggaacagaagagcccttcactatgaaagccagctgtggagtaaaattccaaatttagatgacagttttaaaactca
gtttgcagccttaggaggatggggtttgcatcagccacctcctcaaatatttttaaaaatattaccacaaagtgggccaa
ttggaggtattaaatcaatgggaattactaccttagttcagtatgccgtgggaattatgacagtaaccatgacatttaaa
ttgggccccgtaaagctacgggacggtggaatcctcaacctggagtgtatcccccgcacgcagcaggtcatttaccata
tgtactatatgaccccacagctacagatgcaaaacaacaccacagacatggatatgaaaagcctgaagaattgtggacag
ccaaaagccgtgtgcacccattgtaagtcgacatactc

FIG. 6A

B1 VP1 amino acid sequence:

MSKESGKWWESDDKFAKAVYQQFVEFYEKVTGTDLELIQILKDHYNISLDNPL
ENPSSLFDLVARIKNNLKNSPDLYSHHFQSHGQLSDHPHALSSSSSHAEPRGEDAVLSSE
DLHKPGQVSVQLPGTNYVGPGNELQAGPPQSAVDSAARIHDFRYSQLAKLGINPYTHWTV
ADEELLKNIKNETGFQAQVVKDYFTLKGAAAPVAHFQGSLPEVPAYNASEKYPSMTSVNS
AEASTGAGGGGSNPVKSMWSEGATFSANSVTCTFSRQFLIPYDPEHHYKVFSPAASSCHN
ASGKEAKVCTISPIMGYSTPWRYLDFNALNLFFSPLEFQHLIENYGSIAPDALTVTISEI
AVKDVTDKTGGGVQVTDSTTGRLCMLVDHEYKYPYVLGQGQDTLAPELPIWVYFPPQYAY
~~LTVGDVNTQGISGDSKKLASEESAFYVLEHSSFQLLGTGGTATMSYKFPPVPPENLEGCS~~
QHFYEMYNPLYGSRLGVPDTLGGDPKFRSLTHEDHAIQPQNFMPGPLVNSVSTKEGDSSS
TGAGKALTGLSTGTSQNTRISLRPGPVSQPYHHWDTDKYVTGINAISHGQTTYGNAEDKE
YQQGVGRFPNEKEQLKQLQGLNMHTYFPNKGTQQYTDQIERPLMVGSVWNRRALHYESQL
WSKIPNLDDSFKTQFAALGGWGLHQPPPQIFLKILPQSGPIGGIKSMGITTLVQYAVGIM
TVTMTFKLGPRKATGRWNPQPGVYPPHAAGHLPYVLYDPTATDAKQHHRHGYEKPEELWT
AKSRVHPL

FIG. 6B

B1 VP2 single stranded DNA sequence:

atactcaagcttacaaaacaaaatgacttcagttaattctgcagaagccagcactggtgcaggaggggggggcagtaatcctgtgaaaagcatgtggagtgagggggc
cactttagtgccaactctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccatt
ataaggtgttttctcccgcagcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccata
atgggatactcaaccccatggagatatttagattttaatgctttaaatttatttttttcacctttagagtttcagcactt
aattgaaaattatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacggaca
aaactggaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtaccca
tatgtgttagggcaaggtcaagatactttagccccagaacttcctatttgggtatactttccccctcaatacgcttactt
aacagtaggagatgttaacacacaaggaatttctggagacagcaaaaaattggcaagtgaagaatcagcattttatgttt
tggaacacagttcttttcagcttttaggtacaggaggtacagcaactatgtcttataagtttcctccagtgcccccagaa
aatttagagggctgcagtcaacacttttatgaaatgtacaacccccttatacggatcccgcttaggggttcctgacacatt
aggaggtgacccaaaatttagatctttaacacatgaagaccatgcaattcagccccaaaacttcatgccagggccactag
taaactcagtgtctacaaaggagggagacagctctagtactggagctggaaaagccttaacaggccttagcacaggtacc
tctcaaaacactagaatatccttacgccctgggccagtgtctcagccgtaccaccactgggacacagataaatatgtcac
aggaataaatgccatttctcatggtcagaccacttatggtaacgctgaagacaaagagtatcagcaaggagtgggtagat
ttccaaatgaaaaagaacagctaaaacagttacagggtttaaacatgcacacctactttcccaataaaggaacccagcaa
tatacagatcaaattgagcgcccccctaatggtgggttctgtatggaacagaagagcccttcactatgaaagccagctgtg
gagtaaaattccaaatttagatgacagttttaaactcagtttgcagccttaggaggatggggtttgcatcagccacctc
ctcaaatatttttaaaaatattaccacaaagtgggccaattggaggtattaaatcaatgggaattactaccttagttcag
tatgccgtgggaattatgacagtaaccatgacatttaaattggggccccgtaaagctacgggacggtggaatcctcaacc
tggagtgtatcccccgcacgcagcaggtcatttaccatatgtactatatgaccccacagctacagatgcaaaacaacacc
acagacatggatatgaaaagcctgaagaattgtggacagccaaaagccgtgtgcacccattgtaagtcgacatactc

FIG. 7A

B1 VP2 amino acid sequence:

MTSVNSAEASTGAGGGGSNPVKSMWSEGATFSANSVTCTFSRQFLIPYDPEHH
YKVFSPAASSCHNASGKEAKVCTISPIMGYSTPWRYLDFNALNLFFSPLEFQHLIENYGS
IAPDALTVTISEIAVKDVTDKTGGGVQVTDSTTGRLCMLVDHEYKYPYVLGQGQDTLAPE
LPIWVYFPPQYAYLTVGDVNTQGISGDSKKLASEESAFYVLEHSSFQLLGTGGTATMSYK
FPPVPPENLEGCSQHFYEMYNPLYGSRLGVPDTLGGDPKFRSLTHEDHAIQPQNFMPGPL
VNSVSTKEGDSSSTGAGKALTGLSTGTSQNTRISLRPGPVSQPYHHWDTDKYVTGINAIS
HGQTTYGNAEDKEYQQGVGRFPNEKEQLKQLQGLNMHTYFPNKGTQQYTDQIERPLMVGS
VWNRRALHYESQLWSKIPNLDDSFKTQFAALGGWGLHQPPPQIFLKILPQSGPIGGIKSM
GITTLVQYAVGIMTVTMTFKLGPRKATGRWNPQPGVYPPHAAGHLPYVLYDPTATDAKQH
HRHGYEKPEELWTAKSRVHPL

FIG. 7B

B6 NS1 single stranded DNA sequence:

atactcttcgaacaaaacaaaatggagctatttagaggggtgcttcaagtttcttctaatgttctggactgtgctaacgataactggtggtgctctt
tactggatttagacacttctgactgggaaccactaactcatactaacagactaatggcaatatacttaagcagtgtggcttctaagcttgacttta
ctggggggccactagcagggtgcttgtactttttcaagtagaatgtaacaaatttgaagaaggctatcatattcatgtggttattgggggggcca
gggttaaaccccagaaacctcacagtgtgtgtagaggggttatttaatatgtactttatcaccttgtaactgaaaatctgaagctaaaatttttgc
caggaatgactacaaaaggcaaatactttagagatggagagcagtttatagaaaactatttaatgaaaaaaatacctttaaatgttgtatggtgt
gttactaatattgatggacatatagatacctgtatttctgctacttttagaaagggagcttgccatgccaagaaaccccgcatcaccacagccat
aaatgatactagtactgatgctggggagtctagcggcacagggcagaggttgtgccatttaatgggaagggaactaaggctagcataaag
tttcaaactatggtaaactggttgtgtgaaaacagagtgtttacagaggataagtggaaactagttgactttaaccagtacactttactaagcagt
agtcacagtggaagttttcaaattcaaagtgcactaaaactagcaatttataaagcaactaatttagtgcctactagcacatttttattgcatacag
actttgagcaagttatgtgtattaaagacaataaaattgttaaattgttactttgtcaaaactatgaccccctattagtggggcagcatgtgttaaag
tggattgataaaaaatgtggcaagaaaaacacactgtggttttatggaccgccaagtacagggaaaacaaacttggcaatggccattgctaa
aagtgttccagtatatggcatggttaactggaataatgaaaactttccatttaatgatgtagcaggaaaaagcttggtggtctgggatgaaggta
ttattaagtctacaattgtagaagctgcaaaagccattttaggcgggcaacccaccagggtagatcaaaaaatgcgtggaagtgtagctgtgc
ctggagtacccgtggttataaccagcaatggtgacattacttttgttgtaagcgggaacactacaacaactgtacatgctaaagccttaaaaga
gcgcatggtaaagttaaactttactgtaagatgcagccctgacatggggttactaacagaggctgatgtacaacagtggcttacatggtgtaat
gcacaaagctgggaccactatgaaaactgggcaataaactacacttttgatttccctggaattaatgcagatgccctccacccagacctccaa
accaccccaattgtcacagacaccagtatcagcagcagtggtggtgaaagctctgaagaactcagtgaaagcagctttttttaacctcatcacc
ccaggcgcctggaacactgaaaccccgcgctctagtacgcccatccccgggaccagttcaggagaatcatctgtcggaagcccagtttcc
tccgaagttgtagctgcatcgtgggaagaagccttctacacacctttggcagaccagtttcgtgaactgttagttggggttgattatgtgtggga
cggtgtaaggggtttacctgtctgttgtgtgcaacatattaacaatagtggggggaggcttgggactttgtccccattgcattaatgtaggggctt
ggtataatggatggaaatttcgagaatttaccccagatttggtgcgatgtagctgccatgtgggagcttctaatcccttttctgtgctaacctgca
aaaaatgtgcttacctgtctggattgcaaagctttgtagattatgagtaagtcgacatactc

FIG. 8A

B6 NS1 amino acid sequence:

MELFRGVLQVSSNVLDCANDNWWCSLLDLDTSDWEPLTHTNRLMAIYLSSVAS
KLDFTGGPLAGCLYFFQVECNKFEEGYHIHVVIGGPGLNPRNLTVCVEGLFNNVLYHLVT
ENLKLKFLPGMTTKGKYFRDGEQFIENYLMKKIPLNVVWCVTNIDGHIDTCISATFRKGA
CHAKKPRITTAINDTSTDAGESSGTGAEVVPFNGKGTKASIKFQTMVNWLCENRVFTEDK
WKLVDFNQYTLLSSSHSGSFQIQSALKLAIYKATNLVPTSTFLLHTDFEQVMCIKDNKIV
KLLLCQNYDPLLVGQHVLKWIDKKCGKKNTLWFYGPPSTGKTNLAMAIAKSVPVYGMVNW
NNENFPFNDVAGKSLVVWDEGIIKSTIVEAAKAILGGQPTRVDQKMRGSVAVPGVPVVIT
SNGDITFVVSGNTTTTVHAKALKERMVKLNFTVRCSPDMGLLTEADVQQWLTWCNAQSWD
HYENWAINYTFDFPGINADALHPDLQTTPIVTDTSISSSGGESSEELSESSFFNLITPGA
WNTETPRSSTPIPGTSSGESSVGSPVSSEVVAASWEEAFYTPLADQFRELLVGVDYVWDG
VRGLPVCCVQHINNSGGGLGLCPHCINVGAWYNGWKFREFTPDLVRCSCHVGASNPFSVL
TCKKCAYLSGLQSFVDYE

FIG. 8B

B6 VP1 single stranded DNA sequence:

atactcaagcttacaaaacaaaatgagtaaagaaagtggcaaatggtgggaaagtgatgataaatttgctaaagctgtgtatcagcaatttgtg
gaattttatgaaaaggttactggaacagacttagagcttattcaaatattaaaagatcattataatatttctttagataatcccctagaaaacccatc
ctctttgtttgacttagttgctcgtattaaaaataaccttaaaaactctccagacttatatagtcatcattttcaaagtcatggacagttatctgaccac
ccccatgccttatcatccagtagcagtcatgcagaacctagaggagaagatgcagtattatctagtgaagacttacacaagcctgggcaagtt
agcgtacaactacccggtactaactatgttgggcctggcaatgagctacaagctgggcccccgcaaagtgctgttgacagtgctgcaaggat
tcatgactttaggtatagccaactggctaagttgggaataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaa
tgaaactgggtttcaagcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgccggaa
gttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcactggtgcaggagggggggggcagta
atcctgtgaaaagcatgtggagtgagggggccacttttagtgccaactctgtaacttgtacattttccagacaattttttaattccatatgacccag
agcaccattataaggtgttttctcccgcagcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgg
gatactcaaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaattatggaagtatagct
cctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacaaacaaaactggaggggggggtgcaggttactgacagcactaca
gggcgcctatgcatgttagtagaccatgaatataagtacccatatgtgttagggcaaggtcaagatactttagccccagaacttcctatttgggt
atactttcccccctcaatacgcttacttaacagtaggagatgttaacacacaaggaatttctggagacagcaaaaaattggcaagtgaagaatca
gcattttatgttttggaacacagttcttttcagcttttaggtacaggaggtacagcaactatgtcttataagtttcctccagtgcccccagaaaattt
agagggctgcagtcaacacttttatgaaatgtacaaccccttatacggatcccgcttaggggttcctgacacattaggaggtgacccaaaattt
agatctttaacacatgaagaccatgcaattcagccccaaaacttcatgccagggccactagtaaactcagtgtctacaaaggagggagacag
ctctagtactggagctggaaaagccttaacaggccttagcacaggtacctctcaaaacactagaatatccttacgccctggcccagtgtctca
gccgtaccaccactgggacacagataaatatgtcacaggaataaatgccatttctcatggtcagaccacttatggtaacgctgaagacaaag
agtatcagcaaggagtgggtagatttccaaatgaaaaagaacagctaaaacagttacagggtttaaacatgcacacctactttcccaataaag
gaacccagcaatatacagatcaaattgagcgcccccctaatggtgggttctgtatggaacagaagagcccttcactatgaaagccagctgtgg
agtaaaattccaaatttagatgacagttttaaaactcagtttgcagccttaggaggatggggtttgcatcagccacctcctcaaatattCttaaaa
atattaccacaaagtgggccaa ttggaggtattaaatcaatgggaattactaccttagttcagtatgccgtgggaattatgacagtaaccatga
catttaaa ttggggccccgtaaagctacgggacggtggaatcctcaacctggagtgtatccccccgcacgcagcaggtcatttaccata tgta
ctatatgaccccacagctacagatgcaaaacaacaccacagacatggatatgaaaagcctgaagaattgtggacagccaaaagccgt gtg
cacccattgtaagtcgacatactc

FIG. 9A

B6 VP1 amino acid sequence:

MSKESGKWWESDDKFAKAVYQQFVEFYEKVTGTDLELIQILKDHYNISLDNPL
ENPSSLFDLVARIKNNLKNSPDLYSHHFQSHGQLSDHPHALSSSSSHAEPRGEDAVLSSE
DLHKPGQVSVQLPGTNYVGPGNELQAGPPQSAVDSAARIHDFRYSQLAKLGINPYTHWTV
ADEELLKNIKNETGFQAQVVKDYFTLKGAAAPVAHFQGSLPEVPAYNASEKYPSMTSVNS
AEASTGAGGGGSNPVKSMWSEGATFSANSVTCTFSRQFLIPYDPEHHYKVFSPAASSCHN
ASGKEAKVCTISPIMGYSTPWRYLDFNALNLFFSPLEFQHLIENYGSIAPDALTVTISEI
AVKDVTNKTGGGVQVTDSTTGRLCMLVDHEYKYPYVLGQGQDTLAPELPIWVYFPPQYAY
LTVGDVNTQGISGDSKKLASEESAFYVLEHSSFQLLGTGGTATMSYKFPPVPPENLEGCS
QHFYEMYNPLYGSRLGVPDTLGGDPKFRSLTHEDHAIQPQNFMPGPLVNSVSTKEGDSSS
TGAGKALTGLSTGTSQNTRISLRPGPVSQPYHHWDTDKYVTGINAISHGQTTYGNAEDKE
YQQGVGRFPNEKEQLKQLQGLNMHTYFPNKGTQQYTDQIERPLMVGSVWNRRALHYESQL
WSKIPNLDDSFKTQFAALGGWGLHQPPPQIFLKILPQSGPIGGIKSMGITTLVQYAVGIM
TVTMTFKLGPRKATGRWNPQPGVYPPHAAGHLPYVLYDPTATDAKQHHRHGYEKPEELWT
AKSRVHPL

FIG. 9B

B6 VP2 single stranded DNA sequence:

atactcaagcttacaaaacaaaatgacttcagttaattctgcagaagccagcactggtgcaggagggggggcagtaatcctgtgaaaagcatgtggagtgagggggc
cacttttagtgccaactctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccatt
ataaggtgttttctcccgcagcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccata
atgggatactcaaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcactt
aattgaaaattatggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacaaaca
aaactggaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtaccca
tatgtgttagggcaaggtcaagatactttagccccagaacttcctatttgggtatactttccccctcaatacgcttactt
aacagtaggagatgttaacacacaaggaatttctggagacagcaaaaaattggcaagtgaagaatcagcattttatgttt
tggaacacagttcttttcagcttttaggtacaggaggtacagcaactatgtcttataagtttcctccagtgcccccagaa
aatttagagggctgcagtcaacacttttatgaaatgtacaaccccttatacggatcccgcttaggggttcctgacacatt
aggaggtgacccaaaatttagatctttaacacatgaagaccatgcaattcagccccaaaacttcatgccagggccactag
taaactcagtgtctacaaaggagggagacagctctagtactggagctggaaaagccttaacaggccttagcacaggtacc
tctcaaaacactagaatatccttacgccctgggccagtgtctcagccgtaccaccactgggacacagataaatatgtcac
aggaataaatgccatttctcatggtcagaccacttatggtaacgctgaagacaaagagtatcagcaaggagtgggtagat
ttccaaatgaaaaagaacagctaaaacagttacagggtttaaacatgcacacctactttcccaataaaggaacccagcaa
tatacagatcaaattgagcgcccccctaatggtgggttctgtatggaacagaagagcccttcactatgaaagccagctgtg
gagtaaaattccaaatttagatgacagttttaaaactcagtttgcagccttaggaggatggggtttgcatcagccacctc
ctcaaatatttttaaaaatattaccacaaagtgggccaattggaggtattaaatcaatgggaattactaccttagttcag
tatgccgtgggaattatgacagtaaccatgacatttaaattggggccccgtaaagctacgggacggtggaatcctcaacc
tggagtgtatcccccgcacgcagcaggtcatttaccatatgtactatatgaccccacagctacagatgcaaaacaacacc
acagacatggatatgaaaagcctgaagaattgtggacagccaaaagccgtgtgcacccattgtaagtcgacatactc

FIG. 10A

B6 VP2 amino acid sequence:

MTSVNSAEASTGAGGGGSNPVKSMWSEGATFSANSVTCTFSRQFLIPYDPEHH
YKVFSPAASSCHNASGKEAKVCTISPIMGYSTPWRYLDFNALNLFFSPLEFQHLIENYGS
IAPDALTVTISEIAVKDVTNKTGGGVQVTDSTTGRLCMLVDHEYKYPYVLGQGQDTLAPE
LPIWVYFPPQYAYLTVGDVNTQGISGDSKKLASEESAFYVLEHSSFQLLGTGGTATMSYK
FPPVPPENLEGCSQHFYEMYNPLYGSRLGVPDTLGGDPKFRSLTHEDHAIQPQNFMPGPL
VNSVSTKEGDSSSTGAGKALTGLSTGTSQNTRISLRPGPVSQPYHHWDTDKYVTGINAIS
HGQTTYGNAEDKEYQQGVGRFPNEKEQLKQLQGLNMHTYFPNKGTQQYTDQIERPLMVGS
VWNRRALHYESQLWSKIPNLDDSFKTQFAALGGWGLHQPPPQIFLKILPQSGPIGGIKSM
GITTLVQYAVGIMTVTMTFKLGPRKATGRWNPQPGVYPPHAAGHLPYVLYDPTATDAKQH
HRHGYEKPEELWTAKSRVHPL

FIG. 10B

CH80-1

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgttaaaagcatgtggagtgaggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttgttttttcacctttagagtttcagcatttaattgaaaact
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacagacaaaactg
gagggggagtacaagttactgacagcactaccgggcgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11A

CH81-3

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgttaaaagcatgtggagtgaggggccacttttagtgccaact
ctgtaacttgtacattttccagacagtttttaattccatatgacccagagcaccattataaggtgttttcgcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatacttagattttaatgctttaaatttatttttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacggacaaaactg
gagggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatacaagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11B

B19SCL1-4

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgtgaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacggacaaaactg
gaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11C

B19SCL2-1

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggaggggggggcagtaatcctgtgaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacggacaaaactg
gaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11D

B19SCL3-1

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtgaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
atggaagtatagctcctgatgctttaactgtaaccatatcagaaattgctgttaaggatgttacggacaaaactg
gagggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11E

B19SCL4-3

ataaatccatatactcattggactgtagcagatgaagagcttttaaaaaatataaaaaatgaaactgggtttcaa
gcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgcc
ggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagcac
tggtgcaggagggggggcagtaatcctgtgaaaagcatgtggagtgaggggggccacttttagtgccaac
tctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgca
gcaagtagctgccacaatgccagtggaaaggaggcaaaggtttgcaccattagtcccataatgggatactc
aaccccatggagatatttagattttaatgctttaaatttattttttcacctttagagtttcagcacttaattgaaaatt
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gagggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11F

B19SCL5-2

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gaggggggggtgcaggttactgacagcactacagggcgcctatgcatgttagtagaccatgaatataagtac
ccatatgtgttagggcaaggtcaggatactttag

FIG. 11G

B19SCL6-2

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FIG. 11H

B19SCL7-3

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cccatatgtgttagggcaaggtcaggatactttag

FIG. 11I

B19SCL8-2

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cggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagca
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ctctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgc
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FIG. 11J

B19SCL9-1

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FIG. 11K

B19SCL9-9

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FIG. 11L

B19SCL10-2

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FIG. 11M

B19SCL11-1

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FIG. 11N

B19SCL12-1

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FIG. 11O

B19SCL13-3

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FIG. 11P

B19SCL14-1

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cccatatgtgttagggcaaggtcaggatactttag

FIG. 11Q

B19SCL15-3

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FIG. 11R

B19SCL16-2

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agcacaagtagtaaaagactactttactttaaaaggtgcagctgcccctgtggcccattttcaaggaagtttgc
cggaagttcccgcttacaacgcctcagaaaaatacccaagcatgacttcagttaattctgcagaagccagca
ctggtgcaggagggggggcagtaatcctgtgaaaagcatgtggagtgagggggccacttttagtgccaa
ctctgtaacttgtacattttccagacaatttttaattccatatgacccagagcaccattataaggtgttttctcccgc
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cccatatgtgttagggcaaggtcaggatactttat

FIG. 11S

B19SCL17-1

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FIG. 11T

B19SCL18-1

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FIG. 11U

B19SCL19-1

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FIG. 11V

B19SCL20-3

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FIG. 11W

B19SCL21-3

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FIG. 11X

B19SCL22-11
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FIG. 11Y

B19SCL2-14
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FIG. 11Z

FIGURE 12

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: isolate CH47-26

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gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg ttaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaatttg 480
ttttttcac ctttagagtt tcagcattta attgaaaact atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggagtacaag ttactgacag cactaccggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700
```

<210> 2
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH48-29

<400> 2
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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaacaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggtggc 240
agtaatcctg ccaaaagcat gtggagtgag gggccactt ttactgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag ctagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aactccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcaccta attgaaaatt atggaagtat agctcctgat 540
gatttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgcc tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700
```

<210> 3
<211> 700
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH33-2

<400> 3
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaacaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggtggc 240
agtaatcctg ccaaaagcat gtggagtgag gggccactt ttactgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag ctagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aactccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcaccta attgaaaatt atggaagtat agctcctgat 540
gatttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgct tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 4
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH33-3

<400> 4
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaca 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggtggc 240
agtaatcctg ccaaaagcat gtggagtgag gggccactt ttactgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag ctagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aactccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcaccta attgaaaatt atggaagtat agctcctgat 540
gatttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgcc tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 5
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH33-4

<400> 5
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggtggc 240
agtaatcctg ccaaaagcat gtggagtgag gggccactt ttactgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag ctagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aactccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcaccta attgaaaatt atggaagtat agctcctgat 540
gatttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgcc tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 6
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH42-7

<400> 6
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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccaggcagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700
```

<210> 7
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH42-18

<400> 7
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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700
```

<210> 8
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH42-19

<400> 8
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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
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```
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700

<210> 9
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH46-23

<400> 9
attaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaancaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccaggcagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag atttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700

<210> 10
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH1-1

<400> 10
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag atttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700

<210> 11
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH1-6

<400> 11
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
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agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 12
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH2-8

<400> 12
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 13
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH2-10

<400> 13
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacag aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 14
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH2-11C

<400> 14
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240

agtaatcctg tgaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 15
<211> 699
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH5-13

<400> 15
ctaaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg ttaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaaag aggcaaaggt ttgcactatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggcagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccaa tgtgttaggg caaggtcagg atactttag 699

<210> 16
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH7-22

<400> 16
ataaatccat gtactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg ttaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaatttg 480
tttttttcac ctttagagtt tcagcattta attgaaaact atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggagtacaag ttactgacag cactaccggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 17
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH13-27

<400> 17
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60

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gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaattctg tcaaaagcat gtggagtgag gggccactt ttagtgctaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag cgagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatc 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700
```

<210> 18
<211> 699
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH14-33

<400> 18
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ataaatccat atactcattg gactgtggca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggga 240
gtaatcctgt taaaagcatg tggagtgagg gggccacttt tagtgccaac tctgtaactt 300
gtacattttc cagacagttt ttaattccat atgacccaga gcaccattat aaggtgtttt 360
ctcccgcagc aagtagctgc cacaatgcca gtggaaaaga ggcaaaggtt tgcaccatta 420
gtcccataat gggatactca accccatgga gatatttaga ttttaatgct ttaaatttat 480
ttttttcacc tttagagttt cagcacttaa ttgaaaatta tggtagtata gctcctgatg 540
ctttaactgt aaccatatca gaaattgctg ttaaagatgt tacagacaaa actggagggg 600
gggtacaggt tactgacagc actacagggc gcctatgcat gttagtggac catgaataca 660
agtacccata tgtgttaggg caaggtcagg atactttag                        699
```

<210> 19
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH62-2

<400> 19
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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcaat taattctgca gaagccagca ctggtgcagg aggggggggc 240
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tctcccgcag ccagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
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gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggc cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700
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<210> 20
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH64-2

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cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg ttaaaagcat gtggagtgag gggcccactt ttagtgccaa ctctgtaact 300
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tcgcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatacttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 21
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH67-2

<400> 21
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cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
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agtaatcctg ttaaaagcat gtggagtgag gggcccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaaag aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaagatg ttacagacaa aactggaggg 600
ggggtacagg ttactgacag cactacaggg cgcctatgca tgttagtgga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 22
<211> 4678
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 4.7 kbp PCR fragment
from parvovirus B19 clone 2-B1

<400> 22
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tgcacccatt gtaaacactc ccaccgtgc cctcagccag gatgtgtaac taaacgccca 4620
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<210> 23

<211> 4678
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 4.7 kbp PCR fragment from parvovirus B19 clone 2-B6

<400> 23
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cttaagcagt gtggcttcta agcttgactt tactgggggg ccactagcag ggtgcttgta 420
ctttttttcaa gtagaatgta acaaatttga agaaggctat catattcatg tggttattgg 480
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<210> 24
<211> 2049
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: NS1 from
parvovirus B19 clone 2-B1

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gacatactc 2049

<210> 25
<211> 671
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: NS1 amino acid from
parvovirus B19 clone 2-B1

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1 5 10 15

Cys Ala Asn Asp Asn Trp Trp Cys Ser Leu Leu Asp Leu Asp Thr Ser
20 25 30

Asp Trp Glu Pro Leu Thr His Thr Asn Arg Leu Met Ala Ile Tyr Leu
35 40 45

Ser Ser Val Ala Ser Lys Leu Asp Phe Thr Gly Gly Pro Leu Ala Gly
50 55 60

Cys Leu Tyr Phe Phe Gln Val Glu Cys Asn Lys Phe Glu Glu Gly Tyr
65 70 75 80

His Ile His Val Val Ile Gly Gly Pro Gly Leu Asn Pro Arg Asn Leu
85 90 95

Thr Val Cys Val Glu Gly Leu Phe Asn Asn Val Leu Tyr His Leu Val
100 105 110

Thr Glu Asn Leu Lys Leu Lys Phe Leu Pro Gly Met Thr Thr Lys Gly
115 120 125

Lys Tyr Phe Arg Asp Gly Glu Gln Phe Ile Glu Asn Tyr Leu Met Lys
130 135 140

Lys Ile Pro Leu Asn Val Val Trp Cys Val Thr Asn Ile Asp Gly His
145 150 155 160

Ile Asp Thr Cys Ile Ser Ala Thr Phe Arg Lys Gly Ala Cys His Ala
165 170 175

Lys Lys Pro Arg Ile Thr Thr Ala Ile Asn Asp Thr Ser Thr Asp Ala
180 185 190

Gly Glu Ser Ser Gly Thr Gly Ala Glu Val Val Pro Phe Asn Gly Lys
195 200 205

Gly Thr Lys Ala Ser Ile Lys Phe Gln Thr Met Val Asn Trp Leu Cys
210 215 220

Glu Asn Arg Val Phe Thr Glu Asp Lys Trp Lys Leu Val Asp Phe Asn
225 230 235 240

Gln Tyr Thr Leu Leu Ser Ser Ser His Ser Gly Ser Phe Gln Ile Gln
245 250 255

Ser Ala Leu Lys Leu Ala Ile Tyr Lys Ala Thr Asn Leu Val Pro Thr
260 265 270

Ser Thr Phe Leu Leu His Thr Asp Phe Glu Gln Val Met Cys Ile Lys
275 280 285

Asn Asn Lys Ile Val Lys Leu Leu Leu Cys Gln Asn Tyr Asp Pro Leu
290 295 300

Leu Val Gly Gln His Val Leu Lys Trp Ile Asp Lys Lys Cys Gly Lys
305 310 315 320

Lys Asn Thr Leu Trp Phe Tyr Gly Pro Pro Ser Thr Gly Lys Thr Asn
325 330 335

Leu Ala Met Ala Ile Ala Lys Ser Val Pro Val Tyr Gly Met Val Asn
340 345 350

Trp Asn Asn Glu Asn Phe Pro Phe Asn Asp Val Ala Gly Lys Ser Leu
355 360 365

Val Val Trp Asp Glu Gly Ile Ile Lys Ser Thr Ile Val Glu Ala Ala
370 375 380

Lys Ala Ile Leu Gly Gly Gln Pro Thr Arg Val Asp Gln Lys Met Arg
385 390 395 400

Gly Ser Val Ala Val Pro Gly Val Pro Val Val Ile Thr Ser Asn Gly
405 410 415

Asp Ile Thr Phe Val Val Ser Gly Asn Thr Thr Thr Thr Val His Ala
420 425 430

Lys Ala Leu Lys Glu Arg Met Val Lys Leu Asn Phe Thr Val Arg Cys
435 440 445

Ser Pro Asp Met Gly Leu Leu Thr Glu Ala Asp Val Gln Gln Trp Leu
450 455 460

Thr Trp Cys Asn Ala Gln Ser Trp Asp His Tyr Glu Asn Trp Ala Ile
465 470 475 480

Asn Tyr Thr Phe Asp Phe Pro Gly Ile Asn Ala Asp Ala Leu His Pro
485 490 495

Asp Leu Gln Thr Thr Pro Ile Val Thr Asp Thr Ser Ile Ser Ser Ser
500 505 510

Gly Gly Glu Ser Ser Glu Glu Leu Ser Glu Ser Ser Phe Phe Asn Leu
515 520 525

Ile Thr Pro Gly Ala Trp Asn Thr Glu Thr Pro Arg Ser Ser Thr Pro
530 535 540

Ile Pro Gly Thr Ser Ser Gly Glu Ser Ser Val Gly Ser Pro Val Ser
545 550 555 560

Ser Glu Val Val Ala Ala Ser Trp Glu Glu Ala Phe Tyr Thr Pro Leu
565 570 575

Ala Asp Gln Phe Arg Glu Leu Leu Val Gly Val Asp Tyr Val Trp Asp
580 585 590

Gly Val Arg Gly Leu Pro Val Cys Cys Val Gln His Ile Asn Asn Ser
595 600 605

Gly Gly Gly Leu Gly Leu Cys Pro His Cys Ile Asn Val Gly Ala Trp
610 615 620

Tyr Asn Gly Trp Lys Phe Arg Glu Phe Thr Pro Asp Leu Val Arg Cys
625 630 635 640

Ser Cys His Val Gly Ala Ser Asn Pro Phe Ser Val Leu Thr Cys Lys
645 650 655

Lys Cys Ala Tyr Leu Ser Gly Leu Gln Ser Phe Val Asp Tyr Glu
660 665 670

<210> 26
<211> 2380
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP1 from
parvovirus B19 clone 2-B1

<400> 26
atactcaagc ttacaaaaca aaatgagtaa agaaagtggc aaatggtggg aaagtgatga 60
taaatttgct aaagctgtgt atcagcaatt tgtggaattt tatgaaaagg ttactggaac 120
agacttagag cttattcaaa tattaaaaga tcattataat atttctttag ataatcccct 180
agaaaaccca tcctctttgt ttgacttagt tgctcgtatt aaaaataacc ttaaaaactc 240
tccagactta tatagtcatc attttcaaag tcatggacag ttatctgacc acccccatgc 300
cttatcatcc agtagcagtc atgcagaacc tagaggagaa gatgcagtat tatctagtga 360
agacttacac aagcctgggc aagttagcgt acaactaccc ggtactaact atgttgggcc 420
tggcaatgag ctacaagctg ggcccccgca aagtgctgtt gacagtgctg caaggattca 480
tgactttagg tatagccaac tggctaagtt gggaataaat ccatatactc attggactgt 540
agcagatgaa gagcttttaa aaaatataaa aaatgaaact gggtttcaag cacaagtagt 600
aaaagactac tttactttaa aaggtgcagc tgcccctgtg gcccattttc aaggaagttt 660
gccggaagtt cccgcttaca acgcctcaga aaaataccca agcatgactt cagttaattc 720
tgcagaagcc agcactggtg caggaggggg gggcagtaat cctgtgaaaa gcatgtggag 780
tgaggggggcc acttttagtg ccaactctgt aacttgtaca ttttccagac aattttttaat 840
tccatatgac ccagagcacc attataaggt gttttctccc gcagcaagta gctgccacaa 900
tgccagtgga aaggaggcaa aggtttgcac cattagtccc ataatgggat actcaacccc 960
atggagatat ttagatttta atgctttaaa tttatttttt tcacctttag agtttcagca 1020
cttaattgaa aattatggaa gtatagctcc tgatgcttta actgtaacca tatcagaaat 1080
tgctgttaag gatgttacgg acaaaactgg aggggggggtg caggttactg acagcactac 1140
agggcgccta tgcatgttag tagaccatga atataagtac ccatatgtgt tagggcaagg 1200
tcaagatact ttagccccag aacttcctat ttgggtatac tttcccccctc aatacgctta 1260
cttaacagta ggagatgtta acacacaagg aatttctgga gacagcaaaa aattggcaag 1320
tgaagaatca gcattttatg ttttggaaca cagttctttt cagcttttag gtacaggagg 1380
tacagcaact atgtcttata agtttcctcc agtgccccca gaaaatttag agggctgcag 1440
tcaacacttt tatgaaatgt acaacccctt atacggatcc cgcttagggg ttcctgacac 1500
attaggaggt gacccaaaat ttagatcttt aacacatgaa gaccatgcaa ttcagcccca 1560
aaacttcatg ccagggccac tagtaaactc agtgtctaca aaggagggag acagctctag 1620
tactggagct ggaaaagcct taacaggcct tagcacaggt acctctcaaa acactagaat 1680
atccttacgc cctgggccag tgtctcagcc gtaccaccac tgggacacag ataaatatgt 1740
cacaggaata aatgccattt ctcatggtca gaccacttat ggtaacgctg aagacaaaga 1800
gtatcagcaa ggagtgggta gatttccaaa tgaaaaagaa cagctaaaac agttacaggg 1860
tttaaacatg cacacctact ttcccaataa aggaacccag caatatacag atcaaattga 1920
gcgcccccta atggtgggtt ctgtatggaa cagaagagcc cttcactatg aaagccagct 1980
gtggagtaaa attccaaatt tagatgacag ttttaaaact cagtttgcag ccttaggagg 2040

atggggtttg catcagccac ctcctcaaat atttttaaaa atattaccac aaagtgggcc 2100
aattggaggt attaaatcaa tgggaattac taccttagtt cagtatgccg tgggaattat 2160
gacagtaacc atgacattta aattggggcc ccgtaaagct acgggacggt ggaatcctca 2220
acctggagtg tatccccccgc acgcagcagg tcatttacca tatgtactat atgaccccac 2280
agctacagat gcaaaacaac accacagaca tggatatgaa aagcctgaag aattgtggac 2340
agccaaaagc cgtgtgcacc cattgtaagt cgacatactc 2380

<210> 27
<211> 781
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP1 amino acid from parvovirus B19 clone 2-B1

<400> 27
Met Ser Lys Glu Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala
1 5 10 15

Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly
20 25 30

Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser
35 40 45

Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala
50 55 60

Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His
65 70 75 80

Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser
85 90 95

Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asp Ala Val Leu Ser Ser
100 105 110

Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr
115 120 125

Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser
130 135 140

Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu
145 150 155 160

Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu
165 170 175

Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val
180 185 190

Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His
195 200 205

Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys
210 215 220

Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala
225 230 235 240

Gly Gly Gly Gly Ser Asn Pro Val Lys Ser Met Trp Ser Glu Gly Ala

245 250 255

Thr Phe Ser Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu
260 265 270

Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala
275 280 285

Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile
290 295 300

Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn
305 310 315 320

Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu
325 330 335

Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu
340 345 350

Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val
355 360 365

Thr Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr
370 375 380

Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu
385 390 395 400

Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val
405 410 415

Gly Asp Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala
420 425 430

Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu
435 440 445

Leu Gly Thr Gly Gly Thr Ala Thr Met Ser Tyr Lys Phe Pro Pro Val
450 455 460

Pro Pro Glu Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr
465 470 475 480

Asn Pro Leu Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly
485 490 495

Asp Pro Lys Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro
500 505 510

Gln Asn Phe Met Pro Gly Pro Leu Val Asn Ser Val Ser Thr Lys Glu
515 520 525

Gly Asp Ser Ser Ser Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser
530 535 540

Thr Gly Thr Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val
545 550 555 560

Ser Gln Pro Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile
565 570 575

Asn Ala Ile Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys

580 585 590

Glu Tyr Gln Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu
595 600 605

Lys Gln Leu Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly
610 615 620

Thr Gln Gln Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser
625 630 635 640

Val Trp Asn Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys
645 650 655

Ile Pro Asn Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly
660 665 670

Gly Trp Gly Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu
675 680 685

Pro Gln Ser Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr
690 695 700

Leu Val Gln Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys
705 710 715 720

Leu Gly Pro Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val
725 730 735

Tyr Pro Pro His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro
740 745 750

Thr Ala Thr Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro
755 760 765

Glu Glu Leu Trp Thr Ala Lys Ser Arg Val His Pro Leu
770 775 780

<210> 28
<211> 1699
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP2 from parvovirus B19 clone 2-B1

<400> 28
atactcaagc ttacaaaaca aaatgacttc agttaattct gcagaagcca gcactggtgc 60
aggagggggg ggcagtaatc ctgtgaaaag catgtggagt gagggggcca cttttagtgc 120
caactctgta acttgtacat tttccagaca atttttaatt ccatatgacc cagagcacca 180
ttataaggtg ttttctcccg cagcaagtag ctgccacaat gccagtggaa aggaggcaaa 240
ggtttgcacc attagtccca taatgggata ctcaacccca tggagatatt tagattttaa 300
tgctttaaat ttatttttt cacctttaga gtttcagcac ttaattgaaa attatggaag 360
tatagctcct gatgctttaa ctgtaaccat atcagaaatt gctgttaagg atgttacgga 420
caaaactgga gggggggtgc aggttactga cagcactaca gggcgcctat gcatgttagt 480
agaccatgaa tataagtacc catatgtgtt agggcaaggt caagatactt tagccccaga 540
acttcctatt tgggtatact ttcccctca atacgcttac ttaacagtag gagatgttaa 600
cacacaagga atttctggag acagcaaaaa attggcaagt gaagaatcag cattttatgt 660
tttggaacac agttcttttc agcttttagg tacaggaggt acagcaacta tgtcttataa 720
gtttcctcca gtgcccccag aaaatttaga gggctgcagt caacactttt atgaaatgta 780
caacccctta tacggatccc gcttaggggt tcctgacaca ttaggaggtg acccaaaatt 840

```
tagatcttta acacatgaag accatgcaat tcagccccaa aacttcatgc cagggccact 900
agtaaactca gtgtctacaa aggaggggaga cagctctagt actggagctg gaaaagcctt 960
aacaggcctt agcacaggta cctctcaaaa cactagaata tccttacgcc ctgggccagt 1020
gtctcagccg taccaccact gggacacaga taaatatgtc acaggaataa atgccatttc 1080
tcatggtcag accacttatg gtaacgctga agacaaagag tatcagcaag gagtgggtag 1140
atttccaaat gaaaaagaac agctaaaaca gttacagggt ttaaacatgc acacctactt 1200
tcccaataaa ggaacccagc aatatacaga tcaaattgag cgcccccctaa tggtgggttc 1260
tgtatggaac agaagagccc ttcactatga aagccagctg tggagtaaaa ttccaaattt 1320
agatgacagt tttaaaactc agtttgcagc cttaggagga tggggtttgc atcagccacc 1380
tcctcaaata tttttaaaaa tattaccaca aagtgggcca attggaggta ttaaatcaat 1440
gggaattact accttagttc agtatgccgt gggaattatg acagtaacca tgacatttaa 1500
attggggccc cgtaaagcta cgggacggtg gaatcctcaa cctggagtgt atcccccgca 1560
cgcagcaggt catttaccat atgtactata tgaccccaca gctacagatg caaaacaaca 1620
ccacagacat ggatatgaaa agcctgaaga attgtggaca gccaaaagcc gtgtgcaccc 1680
attgtaagtc gacatactc                                              1699
```

<210> 29
<211> 554
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP2 amino acid from
parvovirus B19 clone 2-B1

<400> 29
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Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly
  1               5                  10                  15

Gly Ser Asn Pro Val Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser
             20                  25                  30

Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr
         35                  40                  45

Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys
     50                  55                  60

His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile
 65                  70                  75                  80

Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn
                 85                  90                  95

Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly
            100                 105                 110

Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val
        115                 120                 125

Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser
    130                 135                 140

Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro
145                 150                 155                 160

Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile
                165                 170                 175

Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val
            180                 185                 190

Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu
```

```
        195                 200                 205

Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr
    210                 215                 220

Gly Gly Thr Ala Thr Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu
225                 230                 235                 240

Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu
                245                 250                 255

Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly Asp Pro Lys
            260                 265                 270

Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro Gln Asn Phe
        275                 280                 285

Met Pro Gly Pro Leu Val Asn Ser Val Ser Thr Lys Glu Gly Asp Ser
    290                 295                 300

Ser Ser Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser Thr Gly Thr
305                 310                 315                 320

Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val Ser Gln Pro
                325                 330                 335

Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile Asn Ala Ile
            340                 345                 350

Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys Glu Tyr Gln
        355                 360                 365

Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu Lys Gln Leu
    370                 375                 380

Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly Thr Gln Gln
385                 390                 395                 400

Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser Val Trp Asn
                405                 410                 415

Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys Ile Pro Asn
            420                 425                 430

Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly Gly Trp Gly
        435                 440                 445

Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu Pro Gln Ser
    450                 455                 460

Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr Leu Val Gln
465                 470                 475                 480

Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys Leu Gly Pro
                485                 490                 495

Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val Tyr Pro Pro
            500                 505                 510

His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro Thr Ala Thr
        515                 520                 525

Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro Glu Glu Leu
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530 535 540

Trp Thr Ala Lys Ser Arg Val His Pro Leu
545 550

<210> 30
<211> 2049
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: NS1 from
parvovirus B19 clone 2-B6

<400> 30
atactcttcg aacaaaacaa aatggagcta tttagagggg tgcttcaagt ttcttctaat 60
gttctggact gtgctaacga taactggtgg tgctctttac tggatttaga cacttctgac 120
tgggaaccac taactcatac taacagacta atggcaatat acttaagcag tgtggcttct 180
aagcttgact ttactggggg gccactagca gggtgcttgt actttttca agtagaatgt 240
aacaaatttg aagaaggcta tcatattcat gtggttattg gggggccagg gttaaacccc 300
agaaacctca cagtgtgtgt agaggggtta tttaataatg tactttatca ccttgtaact 360
gaaaatctga agctaaaatt tttgccagga atgactacaa aaggcaaata ctttagagat 420
ggagagcagt ttatagaaaa ctatttaatg aaaaaaatac ctttaaatgt tgtatggtgt 480
gttactaata ttgatggaca tatagatacc tgtatttctg ctacttttag aaagggagct 540
tgccatgcca agaaaccccg catcaccaca gccataaatg atactagtac tgatgctggg 600
gagtctagcg gcacaggggc agaggttgtg ccatttaatg ggaagggaac taaggctagc 660
ataaagtttc aaactatggt aaactggttg tgtgaaaaca gagtgtttac agaggataag 720
tggaaactag ttgactttaa ccagtacact ttactaagca gtagtcacag tggaagtttt 780
caaattcaaa gtgcactaaa actagcaatt tataaagcaa ctaatttagt gcctactagc 840
acatttttat tgcatacaga ctttgagcaa gttatgtgta ttaaagacaa taaaattgtt 900
aaattgttac tttgtcaaaa ctatgacccc ctattagtgg ggcagcatgt gttaaagtgg 960
attgataaaa aatgtggcaa gaaaaacaca ctgtggtttt atggaccgcc aagtacaggg 1020
aaaacaaact tggcaatggc cattgctaaa agtgttccag tatatggcat ggttaactgg 1080
aataatgaaa actttccatt taatgatgta gcaggaaaaa gcttggtggt ctgggatgaa 1140
ggtattatta agtctacaat tgtagaagct gcaaaagcca ttttaggcgg gcaacccacc 1200
agggtagatc aaaaaatgcg tggaagtgta gctgtgcctg gagtacccgt ggttataacc 1260
agcaatggtg acattacttt tgttgtaagc gggaacacta caacaactgt acatgctaaa 1320
gccttaaaag agcgcatggt aaagttaaac tttactgtaa gatgcagccc tgacatgggg 1380
ttactaacag aggctgatgt acaacagtgg cttacatggt gtaatgcaca aagctgggac 1440
cactatgaaa actgggcaat aaactacact tttgatttcc ctggaattaa tgcagatgcc 1500
ctccacccag acctccaaac caccccaatt gtcacagaca ccagtatcag cagcagtggt 1560
ggtgaaagct ctgaagaact cagtgaaagc agctttttta acctcatcac cccaggcgcc 1620
tggaacactg aaaccccgcg ctctagtacg cccatccccg ggaccagttc aggagaatca 1680
tctgtcggaa gcccagtttc ctccgaagtt gtagctgcat cgtgggaaga agccttctac 1740
acacctttgg cagaccagtt tcgtgaactg ttagttgggg ttgattatgt gtgggacggt 1800
gtaaggggtt tacctgtctg ttgtgtgcaa catattaaca atagtggggg aggcttggga 1860
ctttgtcccc attgcattaa tgtaggggct tggtataatg gatggaaatt tcgagaattt 1920
accccagatt tggtgcgatg tagctgccat gtgggagctt ctaatccctt ttctgtgcta 1980
acctgcaaaa aatgtgctta cctgtctgga ttgcaaagct ttgtagatta tgagtaagtc 2040
gacatactc 2049

<210> 31
<211> 671
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: NS1 amino acid from
parvovirus B19 clone 2-B6

<400> 31
Met Glu Leu Phe Arg Gly Val Leu Gln Val Ser Ser Asn Val Leu Asp

1 5 10 15

Cys Ala Asn Asp Asn Trp Trp Cys Ser Leu Leu Asp Leu Asp Thr Ser
20 25 30

Asp Trp Glu Pro Leu Thr His Thr Asn Arg Leu Met Ala Ile Tyr Leu
35 40 45

Ser Ser Val Ala Ser Lys Leu Asp Phe Thr Gly Gly Pro Leu Ala Gly
50 55 60

Cys Leu Tyr Phe Phe Gln Val Glu Cys Asn Lys Phe Glu Glu Gly Tyr
65 70 75 80

His Ile His Val Val Ile Gly Gly Pro Gly Leu Asn Pro Arg Asn Leu
85 90 95

Thr Val Cys Val Glu Gly Leu Phe Asn Asn Val Leu Tyr His Leu Val
100 105 110

Thr Glu Asn Leu Lys Leu Lys Phe Leu Pro Gly Met Thr Thr Lys Gly
115 120 125

Lys Tyr Phe Arg Asp Gly Glu Gln Phe Ile Glu Asn Tyr Leu Met Lys
130 135 140

Lys Ile Pro Leu Asn Val Val Trp Cys Val Thr Asn Ile Asp Gly His
145 150 155 160

Ile Asp Thr Cys Ile Ser Ala Thr Phe Arg Lys Gly Ala Cys His Ala
165 170 175

Lys Lys Pro Arg Ile Thr Thr Ala Ile Asn Asp Thr Ser Thr Asp Ala
180 185 190

Gly Glu Ser Ser Gly Thr Gly Ala Glu Val Val Pro Phe Asn Gly Lys
195 200 205

Gly Thr Lys Ala Ser Ile Lys Phe Gln Thr Met Val Asn Trp Leu Cys
210 215 220

Glu Asn Arg Val Phe Thr Glu Asp Lys Trp Lys Leu Val Asp Phe Asn
225 230 235 240

Gln Tyr Thr Leu Leu Ser Ser Ser His Ser Gly Ser Phe Gln Ile Gln
245 250 255

Ser Ala Leu Lys Leu Ala Ile Tyr Lys Ala Thr Asn Leu Val Pro Thr
260 265 270

Ser Thr Phe Leu Leu His Thr Asp Phe Glu Gln Val Met Cys Ile Lys
275 280 285

Asp Asn Lys Ile Val Lys Leu Leu Leu Cys Gln Asn Tyr Asp Pro Leu
290 295 300

Leu Val Gly Gln His Val Leu Lys Trp Ile Asp Lys Lys Cys Gly Lys
305 310 315 320

Lys Asn Thr Leu Trp Phe Tyr Gly Pro Pro Ser Thr Gly Lys Thr Asn
325 330 335

Leu Ala Met Ala Ile Ala Lys Ser Val Pro Val Tyr Gly Met Val Asn

340 345 350

Trp Asn Asn Glu Asn Phe Pro Phe Asn Asp Val Ala Gly Lys Ser Leu
355 360 365

Val Val Trp Asp Glu Gly Ile Ile Lys Ser Thr Ile Val Glu Ala Ala
370 375 380

Lys Ala Ile Leu Gly Gly Gln Pro Thr Arg Val Asp Gln Lys Met Arg
385 390 395 400

Gly Ser Val Ala Val Pro Gly Val Pro Val Val Ile Thr Ser Asn Gly
405 410 415

Asp Ile Thr Phe Val Val Ser Gly Asn Thr Thr Thr Thr Val His Ala
420 425 430

Lys Ala Leu Lys Glu Arg Met Val Lys Leu Asn Phe Thr Val Arg Cys
435 440 445

Ser Pro Asp Met Gly Leu Leu Thr Glu Ala Asp Val Gln Gln Trp Leu
450 455 460

Thr Trp Cys Asn Ala Gln Ser Trp Asp His Tyr Glu Asn Trp Ala Ile
465 470 475 480

Asn Tyr Thr Phe Asp Phe Pro Gly Ile Asn Ala Asp Ala Leu His Pro
485 490 495

Asp Leu Gln Thr Thr Pro Ile Val Thr Asp Thr Ser Ile Ser Ser Ser
500 505 510

Gly Gly Glu Ser Ser Glu Glu Leu Ser Glu Ser Ser Phe Phe Asn Leu
515 520 525

Ile Thr Pro Gly Ala Trp Asn Thr Glu Thr Pro Arg Ser Ser Thr Pro
530 535 540

Ile Pro Gly Thr Ser Ser Gly Glu Ser Ser Val Gly Ser Pro Val Ser
545 550 555 560

Ser Glu Val Val Ala Ala Ser Trp Glu Glu Ala Phe Tyr Thr Pro Leu
565 570 575

Ala Asp Gln Phe Arg Glu Leu Leu Val Gly Val Asp Tyr Val Trp Asp
580 585 590

Gly Val Arg Gly Leu Pro Val Cys Cys Val Gln His Ile Asn Asn Ser
595 600 605

Gly Gly Gly Leu Gly Leu Cys Pro His Cys Ile Asn Val Gly Ala Trp
610 615 620

Tyr Asn Gly Trp Lys Phe Arg Glu Phe Thr Pro Asp Leu Val Arg Cys
625 630 635 640

Ser Cys His Val Gly Ala Ser Asn Pro Phe Ser Val Leu Thr Cys Lys
645 650 655

Lys Cys Ala Tyr Leu Ser Gly Leu Gln Ser Phe Val Asp Tyr Glu
660 665 670

<210> 32
<211> 2380
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP1 from
parvovirus B19 clone 2-B6

<400> 32
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atactcaagc ttacaaaaca aaatgagtaa agaaagtggc aaatggtggg aaagtgatga 60
taaatttgct aaagctgtgt atcagcaatt tgtggaattt tatgaaaagg ttactggaac 120
agacttagag cttattcaaa tattaaaaga tcattataat atttctttag ataatcccct 180
agaaaaccca tcctctttgt ttgacttagt tgctcgtatt aaaaataacc ttaaaaactc 240
tccagactta tatagtcatc attttcaaag tcatggacag ttatctgacc acccccatgc 300
cttatcatcc agtagcagtc atgcagaacc tagaggagaa gatgcagtat tatctagtga 360
agacttacac aagcctgggc aagttagcgt acaactaccc ggtactaact atgttgggcc 420
tggcaatgag ctacaagctg ggcccccgca aagtgctgtt gacagtgctg caaggattca 480
tgactttagg tatagccaac tggctaagtt gggaataaat ccatatactc attggactgt 540
agcagatgaa gagcttttaa aaaatataaa aaatgaaact gggtttcaag cacaagtagt 600
aaaagactac tttactttaa aaggtgcagc tgcccctgtg gcccattttc aaggaagttt 660
gccggaagtt cccgcttaca acgcctcaga aaaataccca agcatgactt cagttaattc 720
tgcagaagcc agcactggtg caggaggggg gggcagtaat cctgtgaaaa gcatgtggag 780
tgagggggcc acttttagtg ccaactctgt aacttgtaca ttttccagac aatttttaat 840
tccatatgac ccagagcacc attataaggt gttttctccc gcagcaagta gctgccacaa 900
tgccagtgga aaggaggcaa aggtttgcac cattagtccc ataatgggat actcaacccc 960
atggagatat ttagatttta atgctttaaa tttatttttt tcacctttag agtttcagca 1020
cttaattgaa aattatggaa gtatagctcc tgatgcttta actgtaacca tatcagaaat 1080
tgctgttaag gatgttacaa acaaaactgg aggggggggtg caggttactg acagcactac 1140
agggcgccta tgcatgttag tagaccatga atataagtac ccatatgtgt tagggcaagg 1200
tcaagatact ttagccccag aacttcctat ttgggtatac tttcccccctc aatacgctta 1260
cttaacagta ggagatgtta acacacaagg aatttctgga gacagcaaaa aattggcaag 1320
tgaagaatca gcattttatg ttttggaaca cagttctttt cagcttttag gtacaggagg 1380
tacagcaact atgtcttata agtttcctcc agtgccccca gaaaatttag agggctgcag 1440
tcaacacttt tatgaaatgt acaacccctt atacggatcc cgcttagggg ttcctgacac 1500
attaggaggt gacccaaaat ttagatcttt aacacatgaa gaccatgcaa ttcagcccca 1560
aaacttcatg ccagggccac tagtaaactc agtgtctaca aaggagggag acagctctag 1620
tactggagct ggaaaagcct taacaggcct tagcacaggt acctctcaaa acactagaat 1680
atccttacgc cctgggccag tgtctcagcc gtaccaccac tgggacacag ataaatatgt 1740
cacaggaata aatgccattt ctcatggtca gaccacttat ggtaacgctg aagacaaaga 1800
gtatcagcaa ggagtgggta gatttccaaa tgaaaaagaa cagctaaaac agttacaggg 1860
tttaaacatg cacacctact ttcccaataa aggaacccag caatatacag atcaaattga 1920
gcgcccccta atggtgggtt ctgtatggaa cagaagagcc cttcactatg aaagccagct 1980
gtggagtaaa attccaaatt tagatgacag ttttaaaact cagtttgcag ccttaggagg 2040
atggggtttg catcagccac ctcctcaaat attcttaaaa atattaccac aaagtgggcc 2100
aattggaggt attaaatcaa tgggaattac taccttagtt cagtatgccg tgggaattat 2160
gacagtaacc atgacattta aattggggcc ccgtaaagct acgggacggt ggaatcctca 2220
acctggagtg tatcccccgc acgcagcagg tcatttacca tatgtactat atgaccccac 2280
agctacagat gcaaaacaac accacagaca tggatatgaa aagcctgaag aattgtggac 2340
agccaaaagc cgtgtgcacc cattgtaagt cgacatactc                      2380
```

<210> 33
<211> 781
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP1 amino acid from
parvovirus B19 clone 2-B6

<400> 33
```
Met Ser Lys Glu Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala
  1               5                  10                  15
```

Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Glu Lys Val Thr Gly
20 25 30

Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser
35 40 45

Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala
50 55 60

Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His
65 70 75 80

Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser
85 90 95

Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asp Ala Val Leu Ser Ser
100 105 110

Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr
115 120 125

Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser
130 135 140

Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu
145 150 155 160

Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu
165 170 175

Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val
180 185 190

Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His
195 200 205

Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys
210 215 220

Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala
225 230 235 240

Gly Gly Gly Gly Ser Asn Pro Val Lys Ser Met Trp Ser Glu Gly Ala
245 250 255

Thr Phe Ser Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu
260 265 270

Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala
275 280 285

Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile
290 295 300

Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn
305 310 315 320

Ala Leu Asn Leu Phe Glu Ser Pro Leu Glu Phe Gln His Leu Ile Glu
325 330 335

Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu
340 345 350

```
Ile Ala Val Lys Asp Val Thr Asn Lys Thr Gly Gly Gly Val Gln Val
        355                 360                 365

Thr Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr
    370                 375                 380

Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu
385                 390                 395                 400

Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val
                405                 410                 415

Gly Asp Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala
            420                 425                 430

Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu
        435                 440                 445

Leu Gly Thr Gly Gly Thr Ala Thr Met Ser Tyr Lys Phe Pro Pro Val
    450                 455                 460

Pro Pro Glu Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr
465                 470                 475                 480

Asn Pro Leu Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly
                485                 490                 495

Asp Pro Lys Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro
            500                 505                 510

Gln Asn Phe Met Pro Gly Pro Leu Val Asn Ser Val Ser Thr Lys Glu
        515                 520                 525

Gly Asp Ser Ser Ser Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser
    530                 535                 540

Thr Gly Thr Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val
545                 550                 555                 560

Ser Gln Pro Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile
                565                 570                 575

Asn Ala Ile Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys
            580                 585                 590

Glu Tyr Gln Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu
        595                 600                 605

Lys Gln Leu Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly
    610                 615                 620

Thr Gln Gln Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser
625                 630                 635                 640

Val Trp Asn Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys
                645                 650                 655

Ile Pro Asn Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly
            660                 665                 670

Gly Trp Gly Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu
        675                 680                 685
```

Pro Gln Ser Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr
690 695 700

Leu Val Gln Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys
705 710 715 720

Leu Gly Pro Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val
725 730 735

Tyr Pro Pro His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro
740 745 750

Thr Ala Thr Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro
755 760 765

Glu Glu Leu Trp Thr Ala Lys Ser Arg Val His Pro Leu
770 775 780

<210> 34
<211> 1699
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP2 from
parvovirus B19 clone 2-B6

<400> 34
atactcaagc ttacaaaaca aaatgacttc agttaattct gcagaagcca gcactggtgc 60
aggagggggg ggcagtaatc ctgtgaaaag catgtggagt gagggggcca cttttagtgc 120
caactctgta acttgtacat tttccagaca atttttaatt ccatatgacc cagagcacca 180
ttataaggtg ttttctcccg cagcaagtag ctgccacaat gccagtggaa aggaggcaaa 240
ggtttgcacc attagtccca taatgggata ctcaacccca tggagatatt tagattttaa 300
tgctttaaat ttattttttt cacctttaga gtttcagcac ttaattgaaa attatggaag 360
tatagctcct gatgctttaa ctgtaaccat atcagaaatt gctgttaagg atgttacaaa 420
caaaactgga gggggggtgc aggttactga cagcactaca gggcgcctat gcatgttagt 480
agaccatgaa tataagtacc catatgtgtt agggcaaggt caagatactt tagccccaga 540
acttcctatt tgggtatact ttccccctca atacgcttac ttaacagtag gagatgttaa 600
cacacaagga atttctggag acagcaaaaa attggcaagt gaagaatcag cattttatgt 660
tttggaacac agttcttttc agcttttagg tacaggaggt acagcaacta tgtcttataa 720
gtttcctcca gtgcccccag aaaatttaga gggctgcagt caacactttt atgaaatgta 780
caaccccttta tacggatccc gcttaggggt tcctgacaca ttaggaggtg acccaaaatt 840
tagatcttta acacatgaag accatgcaat tcagccccaa aacttcatgc cagggccact 900
agtaaactca gtgtctacaa aggagggaga cagctctagt actggagctg gaaaagcctt 960
aacaggcctt agcacaggta cctctcaaaa cactagaata tccttacgcc ctgggccagt 1020
gtctcagccg taccaccact gggacacaga taaatatgtc acaggaataa atgccatttc 1080
tcatggtcag accacttatg gtaacgctga agacaaagag tatcagcaag gagtgggtag 1140
atttccaaat gaaaaagaac agctaaaaca gttacagggt ttaaacatgc acacctactt 1200
tcccaataaa ggaacccagc aatatacaga tcaaattgag cgcccccctaa tggtgggttc 1260
tgtatggaac agaagagccc ttcactatga aagccagctg tggagtaaaa ttccaaattt 1320
agatgacagt tttaaaactc agtttgcagc cttaggagga tggggtttgc atcagccacc 1380
tcctcaaata tttttaaaaa tattaccaca aagtgggcca attggaggta ttaaatcaat 1440
gggaattact accttagttc agtatgccgt gggaattatg acagtaacca tgacatttaa 1500
attggggccc cgtaaagcta cgggacggtg gaatcctcaa cctggagtgt atcccccgca 1560
cgcagcaggt catttaccat atgtactata tgaccccaca gctacagatg caaaacaaca 1620
ccacagacat ggatatgaaa agcctgaaga attgtggaca gccaaaagcc gtgtgcaccc 1680
attgtaagtc gacatactc 1699

<210> 35
<211> 554
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VP2 amino acid from parvovirus B19 clone 2-B6

<400> 35
Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly
1 5 10 15

Gly Ser Asn Pro Val Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Ser
20 25 30

Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr
35 40 45

Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys
50 55 60

His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile
65 70 75 80

Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn
85 90 95

Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly
100 105 110

Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val
115 120 125

Lys Asp Val Thr Asn Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser
130 135 140

Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro
145 150 155 160

Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile
165 170 175

Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Asp Val
180 185 190

Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu
195 200 205

Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Gln Leu Leu Gly Thr
210 215 220

Gly Gly Thr Ala Thr Met Ser Tyr Lys Phe Pro Pro Val Pro Pro Glu
225 230 235 240

Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu
245 250 255

Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly Asp Pro Lys
260 265 270

Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro Gln Asn Phe
275 280 285

Met Pro Gly Pro Leu Val Asn Ser Val Ser Thr Lys Glu Gly Asp Ser
290 295 300

Ser Ser Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser Thr Gly Thr

305 310 315 320

Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val Ser Gln Pro
325 330 335

Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile Asn Ala Ile
340 345 350

Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys Glu Tyr Gln
355 360 365

Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu Lys Gln Leu
370 375 380

Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly Thr Gln Gln
385 390 395 400

Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser Val Trp Asn
405 410 415

Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys Ile Pro Asn
420 425 430

Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly Gly Trp Gly
435 440 445

Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu Pro Gln Ser
450 455 460

Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr Leu Val Gln
465 470 475 480

Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys Leu Gly Pro
485 490 495

Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val Tyr Pro Pro
500 505 510

His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro Thr Ala Thr
515 520 525

Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro Glu Glu Leu
530 535 540

Trp Thr Ala Lys Ser Arg Val His Pro Leu
545 550

<210> 36
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VP-5

<400> 36
aggaagtttg ccggaagttc 20

<210> 37
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VP-3

<400> 37
gtgctgaaac tctaaaggtg 20

<210> 38
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VP2-5

<400> 38
gacatggata tgaaaagcct gaag 24

<210> 39
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VP2-3

<400> 39
gttgttcata tctggttaag tact 24

<210> 40
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer K-1sp

<400> 40
ataaatccat atactcatt 19

<210> 41
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer K-2sp

<400> 41
ctaaagtatc ctgaccttg 19

<210> 42
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Hicks-5

<400> 42
cccgccttat gcaaatgggc ag 22

<210> 43
<211> 22

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Hicks-3

<400> 43
ttgtgttagg ctgtcttata gg 22

<210> 44
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer NS1-5

<400> 44
atactctcta gacaaaacaa aatggagcta tttagagggg tgcttcaagt ttct 54

<210> 45
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer NS1-3

<400> 45
gagtatgtcg acttactcat aatctacaaa gctttgcaat ccagacag 48

<210> 46
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VP1-5SN

<400> 46
atactcaagc ttacaaaaca aaatgagtaa agaaagtggc aaatggtggg aaagt 55

<210> 47
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VPALL-3

<400> 47
gagtatgtcg acttacaatg ggtgcacacg gcttttggct gtccacaatt c 51

<210> 48
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VP2-5SN

<400> 48
atactcaagc ttacaaaaca aaatgacttc agttaattct gcagaagcca gcact 55

<210> 49
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC1

<400> 49
aaaaaaaaaa aaaaaaaaaa atccttaaca gcaatttctg ata 43

<210> 50
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC2

<400> 50
aaaaaaaaaa aaaaaaaaaa cgccctgtag tgctgtcag 39

<210> 51
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC3

<400> 51
aaaaaaaaaa aaaaaaaaaa tatacccaaa taggaagttc tg 42

<210> 52
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC4

<400> 52
aaaaaaaaaa aaaaaaaaaa taaaatgctg attcttcact tgc 43

<210> 53
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC5

<400> 53
aaaaaaaaaa aaaaaaaaaa tgctgtacct cctgtaccta 40

<210> 54
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC6

<400> 54
aaaaaaaaaa aaaaaaaaaa agccctctaa attttctggg 40

<210> 55
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: VSPC7

<400> 55
aaaaaaaaaa aaaaaaaaaa ctcctaatgt gtcaggaacc 40

<210> 56
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VSA1

<400> 56
aattctaata cgactcacta tagggagaag gccatatact cattggactg t 51

<210> 57
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VSA2

<400> 57
aattctaata cgactcacta tagggagaag gccagagcac cattataa 48

<210> 58
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VSA3

<400> 58
aattctaata cgactcacta tagggagaag gcacaatgcc agtggaaa 48

<210> 59
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VSP2

<400> 59
gtgctgaaac tctaaaggt 19

<210> 60
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer VSP1

<400> 60
ggaggcaaag gtttgca 17

<210> 61
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<222> (1)
<223> where 'c' is modified 5' with fluorescein
phosphoramidite

<220>
<221> misc_feature
<222> (20)
<223> where 't' is modified 3' with DABCYL

<220>
<223> Description of Artificial Sequence: primer VSPPR1

<400> 61
cccatggaga tatttagatt 20

<210> 62
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH80-1

<400> 62
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg ttaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaatttg 480
ttttttttcac ctttagagtt tcagcattta attgaaaact atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggagtacaag ttactgacag cactaccggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 63
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate CH81-3

<400> 63
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240

agtaatcctg ttaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tcgcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatacttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 64
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL1-4

<400> 64
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 65
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL2-1

<400> 65
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 66
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL3-1

<400> 66
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60

gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 67
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL4-3

<400> 67
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 68
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL5-2

<400> 68
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 69
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL6-2

<400> 69
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 70
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL7-3

<400> 70
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 71
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL8-2

<400> 71
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag gttttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 72
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL9-1

<400> 72
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcaat taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag ccagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 73
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL9-9

<400> 73
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 74
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL10-2

<400> 74
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 75
<211> 700

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL11-1

<400> 75
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttat 700

<210> 76
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL12-1

<400> 76
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tcaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtgact 300
tgtacatttt ccagacagtt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtccgataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacagacaa aactggaggg 600
ggggtgcaag ttactgacag cagtacaggg cgcctatgca tgttagtaga ccatgaatac 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 77
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL13-3

<400> 77
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtgcatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 78
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL14-1

<400> 78
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 79
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL15-3

<400> 79
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 80
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL16-2

<400> 80
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg agggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600

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ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttat                       700
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<210> 81
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL17-1

<400> 81

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ataaatccat atacttattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700
```

<210> 82
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL18-1

<400> 82

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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
ttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag                       700
```

<210> 83
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL19-1

<400> 83

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ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
```

agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 84
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL20-3

<400> 84
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 85
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL21-3

<400> 85
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag gggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 86
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL22-11

<400> 86
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcggg aggggggggc 240

agtaatcctg tgaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatttag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 87
<211> 700
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: isolate B19SCL2-14

<400> 87
ataaatccat atactcattg gactgtagca gatgaagagc ttttaaaaaa tataaaaaat 60
gaaactgggt ttcaagcaca agtagtaaaa gactacttta ctttaaaagg tgcagctgcc 120
cctgtggccc attttcaagg aagtttgccg gaagttcccg cttacaacgc ctcagaaaaa 180
tacccaagca tgacttcagt taattctgca gaagccagca ctggtgcagg aggggggggc 240
agtaatcctg tgaaaagcat gtggagtgag ggggccactt ttagtgccaa ctctgtaact 300
tgtacatttt ccagacaatt tttaattcca tatgacccag agcaccatta taaggtgttt 360
tctcccgcag caagtagctg ccacaatgcc agtggaaagg aggcaaaggt ttgcaccatt 420
agtcccataa tgggatactc aaccccatgg agatatctag attttaatgc tttaaattta 480
tttttttcac ctttagagtt tcagcactta attgaaaatt atggaagtat agctcctgat 540
gctttaactg taaccatatc agaaattgct gttaaggatg ttacggacaa aactggaggg 600
ggggtgcagg ttactgacag cactacaggg cgcctatgca tgttagtaga ccatgaatat 660
aagtacccat atgtgttagg gcaaggtcag gatactttag 700

<210> 88
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Vpara 8

<400> 88
tccatatgac ccagagcacc a 21

<210> 89
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer Vpara 9

<400> 89
tttccactgg cattgtggc 19

<210> 90
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<222> (1)
<223> where 'a' is modified 5' with Fam

<220>
<221> misc_feature
<222> (21)
<223> where 'g' is modified 3' with Tamra

<220>
<223> Description of Artificial Sequence: primer Vpara10

<400> 90
agctagacct gcatgtcact g 21

<210> 91
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: target sequence

<400> 91
ctacttgctg cgggagaaaa acacct 26

<210> 92
<211> 681
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: internal control
sequence

<400> 92
gaattcactt gtacattttc cagacaattt ttaattccat atgacccaga gcaccattat 60
acagtgacat gcaggtctag ctctgccaca atgccagtgg aaaggaggca aaggtttgca 120
ccattagtcc cataatggga tactcaaccc catggagata tttagatttt aatgctttaa 180
atttattttt ttcaccttta gagtttcagc acttaattga aaattatgga agtatagctc 240
ctgatgcttt aactgtaacc atatcagaaa ttgctgttaa ggatgttacg gacaaaactg 300
gaggggggt gcaggttact gacagcacta cagggcgcct atgcatgtta gtagaccatg 360
aatataagta cccatatgtg ttagggcaag gtcaagatac tttagcccca gaacttccta 420
tttgggtata ctttcccct caatacgctt acttaacagt aggagatgtt aacacacaag 480
gaatttctgg agacagcaaa aaattggcaa gtgaagaatc agcattttat gttttggaac 540
acagttcttt tcagctttta ggtacaggag gtacagcaac tatgtcttat aagtttcctc 600
cagtgccccc agaaaattta gagggctgca gtcaacactt ttatgaaatg tacaacccct 660
tatacggatc ccgctgtcga c 681